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MOSQUITO STUDIES (Diptera, Culicidae)

XXVI. Winter biology of *Culex tarsalis* in Imperial Valley, California

by
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CONTENTS

ABSTRACT 1
INTRODUCTION 2
BACKGROUND 3
  Bionomics 3
  Vector Relationships 4
  Overwintering of Arboviruses 5
METHODOLOGY AND PRELIMINARY STUDIES 6
  Study Area 6
  Sampling Procedure 7
    Collecting methods 8
      Light traps with CO_2 attractant 8
      CO_2 traps 8
      Shelters 9
      Larval collections 10
    Sources of error 10
      Error due to sampling procedure 10
      Bias due to behavior of mosquitoes 10
        Shelter preference 10
        Dispersal 12
  Physiological Age-grading 12
    Criteria 12
    Dissections 14
    Sources of error 15
      Gonotrophic dissociation 15
      Autogeny 15
  Analysis of Data 19
RESULTS AND DISCUSSION 20
  Weather 20
  Population Density 21
    Mobility 21
    Females 22
    Males 26
    Immatures 27
  Physiological Parameters 29
    Fat body development 29
    Insemination 31
    Blood feeding and ovarian development 31
    Parity 33
CONCLUSIONS 34
REFERENCES CITED 35
FIGURES 43

MOSQUITO STUDIES (Diptera, Culicidae)

XXVI. WINTER BIOLOGY OF *CULEX TARSALIS* IN IMPERIAL VALLEY, CALIFORNIA[1]

by

Michael J. Nelson[2]

ABSTRACT

Culex tarsalis Coquillett, 1896 is the most important vector of the viruses of western equine encephalomyelitis and St. Louis encephalitis in the western United States during the summer, but the overwintering mechanism for these 2 viruses is unknown. In most areas, *tarsalis* cannot carry virus through the winter because blood-fed females do not survive the coldest months. Overwintering females develop a large fat body and experience a developmental diapause. They usually do not take their first blood meal until the late winter or early spring. However, the mild winters in the Imperial Valley of southern California are more conducive to mosquito activity.

From September 1967 until April 1970, a population of *tarsalis* was studied at the Wister Unit of the Imperial Waterfowl Management Area, Imperial County, California, to determine to what degree feeding and other activities were maintained throughout the year. Each month, adult mosquitoes were collected in light traps with CO_2 attractants, CO_2 bait can traps, and resting shelters. Monthly searches for breeding sites were made.

The following characteristics of the females were recorded: external condition, insemination, stage of ovarian development, presence or absence of muscle tissue remnants, meconium, vertebrate blood, fat body, coiled ovarian tracheoles, and follicular relics. The degree of error in the interpretation of these age-grading criteria due to autogeny was similar to that of comparable studies of the winter biology of *tarsalis* in the Central Valley of California.

There were 2 depressions of the population density each year: a major one in the winter and a lesser in the summer. In contrast to studies to the north, at Wister during every winter month females were attracted to CO_2, males were found in shelters, and pupae and larvae of all instars were collected. Fat body developed only slightly and was largely exhausted before the coldest days of winter. The percentages

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of blood-engorged, gravid, and parous females decreased in the autumn, but recovered in December.

In the Imperial Valley of California, winter diapause of *tarsalis* is very weakly expressed. The duration of diapause is different at the various latitudes within the range of this species. A combination of short photoperiod and low temperature apparently initiates diapause at all latitudes. The relatively high fall and winter temperatures in the Imperial Valley are probably largely responsible for the weak expression and short duration of diapause there.

Potentially, *tarsalis* is a good candidate for overwintering arboviruses in the Imperial Valley. However, other workers have thus far failed to isolate the viruses from this species or from vertebrates in December and January at Wister. Without direct evidence of the viruses in the winter, it is not possible to determine the population density of mosquitoes and the level of blood feeding necessary to maintain the cycle of transmission.

INTRODUCTION

Culex tarsalis Coquillett, 1896 is the most important vector of western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) in the western United States. Although the mosquito-avian transmission cycle of these viruses has been well documented for the summer months, the overwintering mechanism is unknown. In most areas, *tarsalis* cannot overwinter virus because blood-fed females do not survive the coldest months. However, the mild winters in the Imperial Valley of southern California are more conducive to mosquito activity.

In August 1967, following a report in June of 2 horse cases of WEE in Imperial County, Dr. Telford H. Work and I made a preliminary mosquito survey of the area. The Wister Unit of the Imperial Wildlife Management Area (p. 7) appeared to be a potentially favorable area for virus activity, as large populations of both mosquitoes and birds were present. Monthly mosquito collections and virus isolation attempts were initiated in September 1967. It soon became evident that, contrary to the behavior of *tarsalis* in other study areas, the Wister population remained relatively active throughout the winter. We speculated that if blood feeding continued at high level throughout the year, a mosquito-avian cycle could continue without interruption, and in the spring migrating birds might carry the virus northward to areas from which it disappeared each winter. Therefore, I initiated a 33-month study to determine to what degree *tarsalis* continued to feed and to maintain other activities throughout the year. Seasonal fluctuations in the following aspects of its biology were investigated and compared to similar studies in other areas:

I. Population density
  A. Mobility
  B. Females
  C. Males
  D. Immatures

II. Physiological parameters
  A. Fat body development
  B. Insemination
  C. Blood feeding and ovarian development
  D. Oviposition

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BACKGROUND

BIONOMICS

Culex tarsalis occurs in the western, central and southern United States, in western Canada north to Northwest Territories (Carpenter and LaCasse, 1955:296) and in Mexico south to the state of Chiapas (Vargas, 1956:27). Jenkins cites collections up to 2700 meters in Utah (1950:914).

Normally, a raft of 200-300 eggs is deposited on the surface of the breeding water and hatching takes place after 2 or 3 days (Brennan and Harwood, 1953:156). The immature stages are found in a variety of habitats, most commonly in still ground pools in hot, irrigated valleys. The breeding water may range from fresh to very foul and usually contains much organic matter (Carpenter and LaCasse, 1955:296; Jenkins, 1950:914; Sjogren, 1968). In the field, larvae survive freezing temperatures (Washino and Bellamy, 1963) and thermal waters at 39° C (Jenkins, 1950:914). The larval stage, consisting of four stadia, lasts only 10 days and the pupal stage 3 days at 24-26° C (Brennan and Harwood, 1953:155-156).

In the summer, females are gonoactive. During the first day or two after emergence they rest, imbibe plant juices and mate. Then a host is sought for a blood meal. Although preferred hosts are homeothermic vertebrates, especially birds (Tempelis et al., 1965), feeding occurs readily on reptiles and amphibians (Gebhardt et al., 1966; Henderson and Senior, 1961) and, in the laboratory, even on caterpillars (Harris et al., 1969). Blood digestion takes about 3 days at 24-27° C, but requires 3 weeks or longer at 10-13° C (Bellamy and Reeves, 1963:320; Rush, Kennedy and Eklund, 1963a:261).

Activities such as flying, mating, host-seeking and feeding occur largely during the twilight hours. During the day, adults rest in protected shelters but will become active and bite if disturbed. Females are capable of dispersing over relatively great distances. Dow, Reeves and Bellamy (1965) showed that marked adults dispersed up to 15 km into a larvicided area, independently of wind direction. Bailey et al. (1965:111) recovered a marked specimen over 24 km downwind from its point of release 2 nights previously. They estimated dispersal distances at 32-40 km.

Longevity is a matter of weeks or months. Bellamy and Reeves (1963:319) reported that some females held at outdoor winter temperatures in San Joaquin Valley, California, survived more than 8 months.

In early fall, the female population is composed of 2 distinct physiological types (Blackmore and Dow, 1962:292; Nelson, 1964:251). The gonoactive group continues to seek hosts, takes blood meals, and oviposit. It is present in ever diminishing numbers, and by late fall almost no individuals of this category are to be found. The females destined to overwinter emerge, mate, feed on plant juices and convert the carbohydrate mainly to lipid in the progressively larger fat body (Harwood and Takata, 1965). These females do not take a blood meal and their ovaries do not develop past the resting stage. They seek protected shelters and are relatively inactive during the winter. Males do not develop a large fat body and do not survive the winter.

Terms such as hibernation, diapause, reproductive diapause, and facultative diapause have been used to describe the winter condition of *tarsalis* females. Arrest of development in mosquitoes may occur in the egg, larval or adult stage, depending on the species (Clements, 1963:220-237). When environment unfavorable for development, such as low temperature, acts directly on an insect and produces torpor only for the duration of the stimulus, it is usually called "quiescence" (Lees, 1955:3). "Diapause" is less dependent on direct influence of unfavorable environment. When development is interrupted in every generation, regardless of external conditions, the term "obligatory diapause" is used. Initiation of diapause may be by indirect effect of the environment, such as decreasing photoperiod, so that some generations do not diapause but others do. This is known as "facultative diapause" (Lees, 1955:5), and Bellamy and Reeves (1963:321) use this term for *tarsalis*. Harwood and Halfhill (1964) and Harwood and Takata (1965) have demonstrated the importance of photoperiod in initiating diapause in *tarsalis*, but low temperature reinforces the effect of shortened day length.

"Hibernation" is often used for the winter reproductive diapause of adult mosquitoes (e.g. Bennington, Sooter and Baer, 1958:299). It is very different from the total arrest of all developmental processes often seen in diapausing embryos or immatures of many insects, where a reactivation period is usually required before cessation of diapause, such as exposure for several weeks to low temperatures, followed by a return to temperature favorable for development. *C. tarsalis* females are moderately active in the winter, unless the temperature is much below freezing (Rush, 1962:180; Bellamy and Reeves, 1963:321) and will feed when transferred to "room" temperature (Rush, 1962:179).

After 2-6 months, depending on the area and the severity of the winter, the overwintered, inseminated, nulliparous females emerge from their shelters, imbibe plant juices, seek a vertebrate host, and take their first blood meal. Early in the season, many females may be attracted to hosts, but the rate of biting is low until the fat body is largely exhausted (Rush and Tempelis, 1967:309). Because of the low ambient temperature, blood digestion and ovarian development are much slower during the first gonotrophic cycle of the overwintered females than later in the season (Rush, Kennedy and Eklund, 1963a:261).

VECTOR RELATIONSHIPS

C. tarsalis is the most important vector of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) in the western United States. Although WEE is usually not fatal to man, the epidemic of 1952 in the Central Valley of California resulted in many human deaths (Longshore et al., 1956:84).

WEE was first isolated from *tarsalis* by Hammon, Reeves, Brookman et al. (1941), and numerous subsequent studies have elucidated its epidemiology. The primary cycle involves *tarsalis* as the vector and wild birds, especially passerines, and domestic fowl as reservoir hosts (Hammon, Reeves and Sather, 1951). Infected mammals, including horses and humans, apparently do not circulate virus in high enough titer to infect mosquitoes, and therefore they are a dead end in the transmission cycle.

After a female mosquito feeds on an infected host, WEE virus invades the midgut cells and replicates. Subsequently it invades almost every other tissue of the body, especially the salivary glands. Although the virus is found in the ovaries of *tarsalis* (Thomas, 1963:159) and has been isolated from males of *Culiseta melanura* (Stamm et al., 1962:74), transovarian transmission is considered unlikely (Reeves, 1961:66; Thomas, 1963:164). The extrinsic incubation period in *tarsalis* can be completed within as short a time as 4 days and a female can transmit the virus for the duration of its life (Barnett, 1956; Bellamy et al., 1967; Thomas, 1963). There have been no indications of pathology in mosquitoes infected with arboviruses (arthropod-borne viruses), except for Semliki Forest virus which causes cytopathology of salivary glands in *Aedes aegypti* (Mims et al., 1966; Lam and Marshall, 1968).

OVERWINTERING OF ARBOVIRUSES

Each winter, many arboviruses, including WEE and SLE, disappear from their vectors and reservoirs, and for many years workers have attempted to determine how the viruses survive the cold months (Reeves, 1958; 1961; 1965).

Possibly, resident birds with chronic latent infections overwinter WEE virus, or migrating birds with short or chronic viremia may reintroduce the virus each spring. Infections usually last a few days (Hammon, Reeves and Sather, 1951:360; Kissling, Chamberlain et al., 1957:49), but Reeves, Hutson et al. (1958) recovered WEE virus from blood and other tissues of several passerine species from 1-10 months after experimental infection. Lord and Calisher (1970) and Stamm and Newman (1963) report southward transport of WEE during fall migrations, but Kissling, Stamm et al. (1957:44) found lower incidence of WEE antibodies in spring migrants than in permanent and winter residents.

Bats have recurrent viremia of SLE virus several weeks after infection (Sulkin, Allen and Sims, 1966:409). Transplacental transmission of virus occurs (Sulkin, Sims and Allen, 1964) and virus and antibodies have been isolated from bats virtually every month of the year (Allen et al., 1970).

WEE has been isolated from 3 genera of snakes, *Thamnophis* (garter snakes), *Pituophis* (gopher snakes) and *Coluber* (racers), and from the frog *Rana pipiens* (Gebhardt, Stanton et al., 1964; Burton et al., 1966). In the laboratory, infected *tarsalis* females transmit the virus to garter snakes (Gebhardt, Stanton and de St. Jeor, 1966), and Thomas and Eklund (1960) showed that snakes inoculated with WEE in the fall could overwinter the virus and transmit it to biting mosquitoes in the spring. Viremia sometimes recurs more than 5 months after initial infection (Burton et al., 1966:1031; Gebhardt and Stanton, 1966:30). Since the virus has been found in the offspring of infected snakes (Gebhardt, Stanton et al., 1964: 173), and both snakes and frogs can be infected orally (Spalatin et al., 1964), many new transmission pathways are feasible: Snakes or frogs may be bitten by infected mosquitoes, they may eat infected mosquitoes, snakes may eat infected frogs, and so on.

The mosquito *Culiseta inornata* (Williston, 1893), a winter species in Imperial

Valley (Chew and Gunstream, 1970:558), is a laboratory vector of WEE (Hammon and Reeves, 1943:433) and harbors the virus in the field (Hammon, Reeves, Benner and Brookman, 1945). However, Washino et al. (1962:270) failed to isolate virus from 4900 females of this species collected mostly in the fall, winter and spring in San Joaquin Valley, California.

In northern Colorado, where winters are severe, 1 winter isolation of western equine encephalomyelitis from *tarsalis* was made from a pool of females collected in a mine on December 30, 1953 (Blackmore and Winn, 1956). However, based on the long and well-defined hibernation of the population in that area, Bennington, Sooter and Baer (1958:304) concluded that *tarsalis* was an unlikely candidate to overwinter the virus. In central Washington, Rush, Brennan and Eklund (1958: 292) failed to isolate WEE virus from overwintering females collected from late September to early April, although many isolations were made in the summer. In eastern Oregon, virus was not found in the cold months, and the earliest isolates from *tarsalis* were in July in one study (Rush, Kennedy and Eklund, 1963b: 286) and not until August in another (Rush and Tempelis, 1967:313). In 13 years of attempts in central North Dakota, the earliest WEE isolation was in June, and in western Idaho, July was the earliest month (Rush, Kennedy and Eklund, 1963a: 260). It is the conclusion of the above workers that *tarsalis* is not a winter vector in the northwestern United States.

Although in San Joaquin Valley, California, diapause is less pronounced, isolates of WEE virus have not been made from November 15 to January 15, the period of minimal blood feeding (Reeves, Bellamy and Scrivani, 1958:82-83). Both WEE virus (Bellamy et al., 1967) and SLE virus (Bellamy et al., 1968) have been experimentally overwintered in *tarsalis*. However, Bellamy and Reeves (1963:322) and Burdick and Kardos (1963:534) concluded that *tarsalis* is not an important overwintering vector of virus because of its winter biology. Nelson (1964:252) speculated that during mild winters this mosquito may occasionally maintain the virus cycle, but that it is probably not the principal overwintering agent.

METHODOLOGY AND PRELIMINARY STUDIES

STUDY AREA

The Imperial Valley is in the southern extreme of California, south of the Salton Sea (fig. 1). Various other studies of *tarsalis* have been made in the Coachella Valley, which is northwest of the Salton Sea, and the Central Valley which is divided into the Sacramento Valley in the north and the San Joaquin Valley in the south.

The Imperial Valley is an irrigated desert of some 2200 km^2 in Imperial County. Water for irrigation of such crops as alfalfa, grains, flax and sugar beets comes primarily from the Colorado River via the All-American Canal and is distributed by a large network of canals. The drains flow into either the Alamo River or New River, both of which empty into the Salton Sea. The Sea is actually an inland lake over 50 km in length, 18 km in width, and approximately 70 m below sea level. It was formed in 1905 when the Colorado River flooded and filled the dry sink. Since water leaves the Salton Sea only by evaporation, the salinity has increased to approximately that of sea water (deStanley, 1966).

The Imperial Waterfowl Management Area was established by the State of California Department of Fish and Game primarily to prevent crop depredation on surrounding farms by migratory waterfowl. It also provides sanctuary for an abundance of wildlife, although hunting is permitted during certain seasons. The Wister Unit of the Area (fig. 2) is on the east shore of the Salton Sea, about 11 km northwest of Niland on State Highway 111. It is about 2 x 6 km, divided into sections by dirt roads, dikes, and canals for irrigation of the marshes. Most irrigation is done during the fall and winter to provide wintering habitat for migratory waterfowl from the north. The 3 main crops, barley, wild millet and alkali bulrush, are harvested each year by the ducks and geese. Cattails are plentiful in the ponds and tamarisk occurs along the borders and in the drier areas with mesquite.

The most common wintering waterfowl are snow goose, Canada goose, pintail duck, green-winged teal and American widgeon. Other common birds are Brewer's blackbird, red-winged blackbird, yellow-headed blackbird, starling, house finch, mourning dove, ground dove, pheasant, Gambel's quail, nighthawk, killdeer, black-necked stilt, avocet and other small shore birds. Common mammals are Audubon cottontail rabbit, black-tailed hare, striped skunk and raccoon. Gopher snakes and rattlesnakes are seen occasionally, frogs are abundant, and several freshwater fish inhabit the ponds, notably *Gambusia affinis*, an efficient predator of mosquito larvae.

Culex tarsalis is the most common mosquito at Wister throughout the year. *Culiseta inornata* is abundant in the winter. *Culex pipiens quinquefasciatus* Say, 1823, *Psorophora confinnis* (Lynch Arribalzaga, 1891), *Aedes vexans* (Meigen, 1830), and *Anopheles franciscanus* McCracken, 1904 are common at other times of the year. *Culex erythrothorax* Dyar, 1907 and *Aedes dorsalis* (Meigen, 1830) are less common.

On the map of Wister (fig. 2), circled numerals mark the sites where the various kinds of collections were made. Sites 1-5, 7, 9 and 10 were on the border of duck ponds that were flooded from about August to March. Sites 6 and 11 were about 0.5 km from the ponds and sites 12 and 13 about 1 km from the ponds. All sites were within a few meters of canals. Sites 7-9 and 11-13 were barley fields where waterfowl fed in the winter.

SAMPLING PROCEDURE

The Wister study was divided into 2 phases: a preliminary phase and a definitive phase. The purpose of the preliminary phase, the 11 lunar months from September 27, 1967 until July 22, 1968, was to determine monthly fluctuations in population density, to gather preliminary data on overwintering activity, and to collect large numbers of mosquitoes for virus isolation attempts done by Dr. Telford H. Work and coworkers at the Division of Infectious and Tropical Diseases, School of Public Health, University of California, Los Angeles. Seven light traps with CO_2 attractant and 7 CO_2 bait can traps were deployed at sites 3-5, 9-11 and 13 (fig. 2) for 3 or 4 consecutive days during the dark phase of the moon each month. Collection dates are given with the population data in tables 5 and 6. On November 30, 1968, use of 3 more light traps and CO_2 traps was initiated at sites 2, 6 and 7. Data from these sites were not included with those from the other 7 sites for estimation of population density for the first 11 months. Routine daytime resting collections from a culvert (site 6) were also initiated in November. Sporadic collections from other culverts and from wooden bridges were made throughout the first 11 months. Occasional larval collections were made.

The definitive phase of the study included the 21 lunar months from August 31, 1968 until April 12, 1970. The emphasis during this period was on the physiological parameters of the population. Again, collections were made each month for 3 days during the dark phase of the moon. Light trap collections were discontinued. CO_2 traps were deployed at sites 2, 6 and 7, and routine shelter collections were made from a culvert (site 6) and from under 4 wooden bridges (sites 1, 2, 5, 7). Routine larval collections were made.

COLLECTING METHODS

Light traps with CO_2 attractant

The CDC (Center for Disease Control, Atlanta, Georgia) miniature light trap (Sudia and Chamberlain, 1962) is a small fan and light assembly mounted in a plastic cylinder. A catching net receives the live insects and an aluminum shade protects them from sun and rain. The trap operates on a 6-volt battery. Phototactic insects are attracted to the light, caught by the suction of the fan and delivered into the catching bag. A block of about 1 kg of dry ice was wrapped in 2 sheets of newspaper, tied with string, and hung next to each light trap at about 1.8 m above the ground (Newhouse, et al., 1966). All traps were deployed before sunset and retrieved shortly after sunrise. Usually, more mosquitoes are attracted to light traps if there are no competing light sources. Although mosquitoes are generally more active during a full moon, more of them are attracted to light traps when there is little or no moonlight (Bidlingmayer, 1967:206). For this reason, I scheduled each collecting trip of 3 or 4 consecutive days at Wister to include the day of the new moon for that month.

The attraction of insects to light is not well understood. One view is that insects utilize a near source of light for a mechanism of orientation which is adapted for distant sources such as the moon (Wigglesworth, 1965:285). While the angle between the line of sight to the moon and the longitudinal axis of the insect remains the same, the insect will fly in a constant compass direction. However, when an insect orients in this manner to a near light source, "The angle of incidence of light can be kept constant only if the insect continually turns towards the source. It will thus move along a logarithmic spiral ending at the light itself..." (Wigglesworth, 1965:285). Another view is expressed by Verheijen (1960). He proposes that animals use light cues of low intensity for orientation at night. A high intensity artificial light source interferes with normal photic orientation, resulting in drifts of the animal toward the light source. Attraction to light varies with species, sex, age and other factors (Southwood, 1966:174-178, 200-201). In my study, light traps were used with CO_2 attractant, which further complicates interpretation of physiological data from mosquitoes collected by this method. For these reasons, use of light traps was discontinued after the preliminary phase of the study. Physiological measurements were made only from females collected in CO_2 traps and shelters.

CO_2 traps

The CO_2 traps (Bellamy and Reeves, 1952) were made from cylindrical "lard cans" (36 cm x 30 cm diam). A circular hole (23 cm diam) was cut in the lid and bottom of each can and replaced by an inwardly pointing, hardware cloth cone. A 2 cm circular opening was left in the apex of each cone. The cans were painted dark red inside and out, and a 1-liter capacity, bottomless, polyethylene cup was fastened to the top of each can on the inside with a strip of sheet metal. The dry ice, prepared in the same manner as for the light traps, was inserted in the cup. Mosquitoes enter the traps through the hole in either cone and are unable to find their way out again. Each trap was suspended from a tree or bush at about 1.5 m above the ground.

Apparently, carbon dioxide activates host-seeking females which then fly upwind to locate the source (Clements, 1963:276). Reeves (1953) found that 3 species of mosquitoes were attracted to CO_2 released from large "stable" traps at rates comparable to those of their preferred hosts. *C. tarsalis*, which feeds on a variety of vertebrates, was attracted to CO_2 released at rates similar to those of chicken, man and horse (or cow). More females were attracted at the higher rate of CO_2 release. Although females of *tarsalis* usually do not bite in the daytime, many attacked the collector when he entered the traps, and mosquitoes were observed probing at the rubber tubing where the gas was released. Of over 8000 adults collected, only 22 were males. In my study only 3 males and 1 engorged female were collected in 444 trap-nights in which 6112 nonengorged females were collected. Therefore, I rejected the possibility that the mosquitoes might be attracted to the traps as daytime resting shelters and considered that the mosquitoes collected in CO_2 traps were seeking hosts. (Although 58 gravid females, each with a very few developing eggs, were collected in the CO_2 traps, I believe that they were also searching for a blood source, p. 18.)

Shelters

A mechanical aspirator (Husbands, 1958) was used to collect mosquitoes from daytime resting shelters. This device was made from a battery-powered vacuum clothes brush that I fitted with a collecting tube (38 cm x 1.27 cm diam) and interchangeable clear plastic cartridges (10 cm x 2.5 cm diam) that held the mosquitoes from each collection. Collections were made at about midday from a cement culvert and 4 wooden bridges. The culvert (fig. 3) consistently contained the largest numbers of mosquitoes. It was a tubular structure (ca. 15 m x 1 m diam) under State Highway 111. It had running or standing water 1-15 cm deep at all times. Mosquitoes were usually distributed about evenly throughout the middle 2/3 of its length. The walls were smooth, and nearly every mosquito could be collected.

The 4 wooden bridges (sites 1, 2, 5, 7) crossed canals at 0.8 km intervals on a dirt road running north to south (fig. 4). Each bridge was supported by wooden beams about 1.5 m above the surface of the slowly moving water in the canal. Mosquitoes rested on the horizontal undersurface of the bridges, on the vertical side of the beams, and on the sloping earth banks. During the first 11 months of the study, all mosquitoes that could be found were collected. However, many parts of the bridges were inaccessible, and even with a flashlight it was difficult to see mosquitoes against the darkly colored, rough wooden and earth surfaces. In August 1968, five 3 gal. (11.4 liter), cylindrical, dark red ice cream cartons were stapled to the southern, interior vertical surface of each bridge between the beams (fig. 5). Collections thereafter were made only from these containers. The red cartons were also placed in various other shaded sites at Wister, but almost no mosquitoes were attracted to them.

Larval collections

Each month, a systematic search for breeding sites was made. A 1-pint (0.473 liter) sauce pan attached to a wooden pole served as the sampling tool. If a breeding site was too shallow for the conventional dipper, a pipette was used to remove all larvae and the mean number of larvae per 0.1 m^2 of water surface was determined. At site 8 (fig. 8), a sewage oxidation pond, routine collections were made from September, 1969 until March, 1970. One dipper of water per 0.1 m^2 of water surface was taken from an area of 6 m^2 of the pond (fig. 9).

SOURCES OF ERROR

There are at least 2 potential sources of error in the data gathered by the methods described above: The first arises from the collecting procedure; the second is introduced by the behavior of the mosquitoes.

Error due to the sampling procedure

The sampling procedure may have been inadequate to describe accurately the Wister population: Collections were made during a very short period each month, samples were not random, and in some months very few mosquitoes were collected.

The monthly collections of 3 or 4 consecutive days were assumed to be representative of the population for the entire lunar month. This probably was not the case when some characteristic of the population was changing rapidly. Temporary aberrant weather surely made some collecting periods very atypical for the month. Biweekly or weekly collections would have been better, and daily collections would have been best, but impractical. In the graphic representations of the various parameters of the population, a line connects the values for each pair of consecutive monthly collecting periods. In this way, seasonal trends can be seen more easily and data from 2 or more collecting methods can be compared on the same graph. However, it must be remembered that each point is an average for 3 or 4 days, not an entire month.

Since samples were taken at the same sites every month, they were not random. The frequency distribution of numbers of mosquitoes per trap per night (trap-night) was not normal. The monthly population means were sometimes influenced by very large or very small numbers of mosquitoes captured at 1 or 2 sites. Standard errors are given (tables 5, 6) to indicate the variability in the numbers of mosquitoes captured, but the standard error cannot be used for statistical tests of differences among the means.

In some months, only a few females were examined for a particular attribute (e.g. fat body development in CO_2 traps and shelters). When sample size (N) was very small (<10), the point on the graph representing the percentage of females with the attribute is bracketed. For all points sample size is given above the graph.

Bias due to the behavior of the mosquitoes

The behavior of *tarsalis* may bias the estimation of physiological parameters in 2 ways: Females may prefer more secluded shelters in the winter, or most of them may disperse to the foothills.

Shelter preference

Overwintering females of *tarsalis* have been found in a variety of shelters. In northern Utah (Dow et al., 1956), northern Colorado (Blackmore and Winn, 1956;

Mail and McHugh, 1961) and western Nebraska (Keener, 1952), they are commonly found in mines and cellars during the coldest months, but some workers are of the opinion that these do not constitute the successful overwintering population. Dow et al. (1956) found that females disappeared from mines in late winter. The temperature in the mines was about 10° C and the mosquitoes remained active, probably exhausting their energy reserves prematurely. Bennington, Blackmore and Sooter (1958:298) collected 2 overwintering females from rodent burrows where the temperature was below 10° C and noted a correspondence of spring emergence with soil temperature inversion. Mail and McHugh (1961) brought overwintering females into the laboratory and found that survival was longest at high humidity and at a temperature of freezing or slightly below. In southern Alberta, Shemanchuk (1965) found occasional wintering females in large animal burrows where the temperature was considerably higher than the ambient temperature but still dropped below freezing nearly every day. He, too, noted a correspondence of spring emergence and soil temperature inversion. In the summer, Harwood and Halfhill (1960) found females and males in vegetation, rock fissures, and animal burrows, but winter females were found almost exclusively in rock piles and talus slopes in central Washington (Harwood, 1962; Rush et al., 1958) and in eastern Oregon (Rush, 1962; Rush et al., 1963b). Harwood (1962) thought that the colder and more stable northfacing talus slopes were most favorable for overwintering adults by reducing activity and energy utilization.

Some workers in Central Valley, California, believe that *tarsalis* prefers "natural" winter shelters to "artificial" ones. Probably, a more useful distinction would be based on the degree of protection from wind and precipitation, and on regimes of temperature, humidity and light intensity. The man-made structures in question are usually more exposed. Mortenson (1953) collected overwintering female *tarsalis* from such natural sites as tree stumps, tree roots, a tree hole, hollow logs, natural wood piles, brush piles, animal burrows, a woodrat's nest, and a rock ledge in San Joaquin Valley, California. He stated that this species was less abundant in "artificial" shelters. Loomis and Green (1955) continued Mortenson's study and concluded that *tarsalis* females preferred "natural" resting shelters to such artificial sites as chicken houses, bridges, and cellers, but their conclusion was based on the percentage of *tarsalis* of all mosquitoes of various species collected rather than on actual numbers, so that preferences of the other species may have biased the results. Ryckman and Arakawa (1952) found *tarsalis* adults in woodrats' nests in late November and early December in Riverside County, but did not find them in all other habitats searched. Kliewer et al. (1969) in Fresno County, California, observed a decrease in the number of *tarsalis* in culverts in winter after an earlier increase, and thought this might be due to a sudden avoidance of these resting sites. They noticed an apparent preference for piles of rocks. Bailey (1965:109) states that many overwintering females are found in man-made structures, but that they prefer natural shelters in the Sacramento Valley.

At Wister, there may well be a winter preference of the females for sites other than cement culverts and wooden bridges. In fact, this may be the explanation for an apparent decrease in the population at these sites in the fall (fig. 15). However, there is no evidence that the winter biology of a hypothetical, inaccessible, subterranean population of females would be different than that of the females in the more open shelters. The winter studies of *tarsalis* in the San Joaquin Valley of Bellamy and Reeves (1963), Burdick and Kardos (1963), Kliewer et al. (1969) and Nelson (1964) were all based on collections from such man-made shelters as culverts, bridges, sheds and wooden boxes, and a definite period of winter diapause was noted. Since the Wister study was based on collections from the same kinds of shelters, the data can be compared to those from the San Joaquin Valley.

Dispersal

Some workers have indicated that in the fall, *tarsalis* disperses from the valleys to pass the winter in the surrounding foothills. In the Sacramento Valley, California, Bailey et al. (1965:109) concluded that there was a definite fall movement into the foothills, especially to the hills downwind from the valley. This dispersal occurred at the same time that the rice fields, the major breeding source, were drained. Abell (1959) studied mosquito populations in the foothills of Fresno County, California, at an intermittent stream that offered plentiful breeding sites from late fall through spring. Twice as many adults were attracted to light traps from mid-October to mid-November as in July. As breeding did not occur in the foothill area in the fall, the adults probably had dispersed there. In a 5-year study in an adjacent canyon, Kliewer et al. (1969:16-17) did not find *tarsalis* adults in any numbers in culverts until late fall. They were abundant throughout the winter. In the valley below, light trap catches were highest in the summer. Since teneral (recently emerged) adults were absent throughout the spring, summer and early fall in the hills, the population probably did not originate there. Indications were that in the autumn the mosquitoes dispersed from the valley to the foothills where they overwintered. In January, they began to feed and returned to the valley without breeding significantly in the foothills.

In the San Joaquin Valley, both the valley and foothill populations of *tarsalis* experience a well defined winter diapause. Therefore, there is no reason to believe that the females remaining at Wister in the winter may be physiologically different from those that may have dispersed to the foothills.

PHYSIOLOGICAL AGE-GRADING

CRITERIA

Females were dissected and the following attributes related to their winter biology and physiological age were recorded: external condition, insemination, muscle remnants, meconium, fat body development, presence of blood, ovarian development, ovarian tracheation and follicular relics.

As a mosquito ages in the field, the external scales and hairs on the body, legs and wings are rubbed off. I divided the degree of external wear into 3 categories, based on the characters used in taxonomic keys to identify *tarsalis* females: (1) **Recently emerged**: Inverted dark-scaled "V"s on venter of each abdominal segment complete; white tarsal bands complete (Carpenter and LaCasse, 1955:269). (2) **Intermediate**: Inverted "V"s unclear; white tarsal bands complete. (3) **Rubbed**: Inverted "V"s nonexistent; white tarsal bands unclear; white scales present on inner margin of antennal torus, distinguishing *tarsalis* from *Culex peus* Speiser, 1904 and other species of *Culex* (Chaniotis and Iltis, 1960).

A female mates once, soon after emergence from the pupa, and the sperm stored in the spermathecae are used to fertilize all the egg batches she produces. Usually, only recently emerged females are noninseminated. Diapausing females are nearly all inseminated.

A meconium, a mass of greenish-brown semisolid substance in the midgut, occurs only in recently emerged adults, as do certain abdominal muscle tissue remnants, but the time required for the disappearance of these 2 physiological age indicators varies considerably with temperature (Rosay, 1961:528).

The fat body of mosquitoes is not a single organ, but a loose meshwork of lobes dispersed throughout the abdomen. It functions as a food storage organ and as an excretory organ where uric acid accumulates. In mosquito larvae, it contains lipid, glycogen and protein, but protein is never deposited in the adult fat body (Clements, 1963:49-51). The glycogen is used mainly for activities such as flying. The lipids appear to be more important for hibernation (Buxton, 1935; Schaefer and Washino, 1969). In diapausing *tarsalis* females, the fat body becomes large and compact in winter and is largely exhausted by spring. Bullock et al. (1959: 184-185) recognized 4 stages of fat body development in *Culex tritaeniorhynchus* Giles, 1901. Their classification was adapted to *tarsalis* by Burdick and Kardos (1963:530). I modified the latter classification for my study: **Class 0.** – No fat cells seen. **Class 1.** – Fat cells around spermathecae only. **Class 2.** – Traces of fat in the abdomen, anterior to the ovaries. **Class 3.** – Abdomen filled with fat body, but not distended. **Class 4.** – Abdomen distended by fat body. Classes 3 and 4 of my classification are approximately the same as Classes 2 and 3, respectively, of Burdick and Kardos (1963:530).

Blood digestion and ovarian development occur simultaneously. The adjective "engorged" is used to describe females with any amount of blood in the midgut. When the blood has been completely digested, the eggs are usually in Stage IV (see below), and the female is "gravid" until the eggs are deposited. Females with neither blood nor developing eggs are "empty".

The reproductive system of *tarsalis* females consists of 2 ovaries and 2 lateral oviducts that join to form a common oviduct. The 2 ovaries contain a variable number of ovarioles (ca. 300). Each ovariole consists of a growth zone anteriorly (germarium) and usually 2 follicles posteriorly. A follicle contains an oocyte and 7 nurse cells. Only 1 egg at a time develops in each ovariole, and the eggs of most ovarioles develop in synchrony after a blood meal. The fully developed eggs pass through the lateral oviduct, are fertilized from the 3 spermathecae attached to the common oviduct, and are deposited in a raft on the water surface.

Christophers (1911) divided the period of ovarian development into 5 phases (Stages I-V). Mer (1936) added Stage N. The following is a brief summary of the classification as I have adapted it to *tarsalis*: **Stage N.** – The 8 cells of the spherical follicle are undifferentiated. **Stage I.** – The oocyte becomes clearly differentiated from the 7 nurse cells. **Stage II.** – Yolk granules appear around the oocyte nucleus. **Stage III.** – Yolk granules obscure the oocyte nucleus and begin to fill the follicle; the follicle becomes broadly oval. **Stage IV.** – The oocyte fills nearly the entire follicle and begins to elongate. **Stage V.** – The egg becomes fully elongate; the micropyle cup becomes distinct. Stage N is seen only in nulliparous females. Stage I is generally thought to be the resting stage for culicine mosquitoes, but Rosay (1969:610) found that either Stage I or Stage II could be the resting stage for some culicine species before their first blood meal, and some anautogenous *tarsalis* females developed Stage II eggs before a subsequent blood meal.

The tightly coiled "skeins" of the ovarian tracheoles of nulliparous (i.e. no history of oviposition) female mosquitoes are irreversibly stretched during development of the ova in the first gonotrophic cycle (Detinova, 1945; 1962:48-51). After oviposition, the ovary returns to approximately its original size, but the uncoiled tracheoles become visible if the ovary is allowed to dry on a microscope slide. This criterion of parity has been evaluated with *tarsalis* females of known history and applied successfully to field populations, but there is generally a portion of the ovaries that either cannot be read or is intermediate between the normal nulliparous and parous conditions (Blackmore and Dow, 1962; Burdick and Kardos, 1963; Kardos and Bellamy, 1961; Nelson, 1964, 1966).

In a like manner, the stomach tracheation has been used to distinguish blood-fed from non-blood-fed females (Detinova, 1962:51-53). Midgut tracheae of nulliparous females form a series of tight bands with coiled tracheoles. After digestion of a blood meal, the tracheae are stretched and the tracheoles are uncoiled. Plant juices do not stretch the stomach tracheae because they first enter the crop and only gradually pass to the stomach. Detinova (1962:52) reports that this criterion is reliable for distinguishing parous from nulliparous females of *Culex pipiens*, but I found that it was inapplicable to *tarsalis* females from a laboratory colony.

In many species of mosquitoes and other haematophagous Diptera, after each oviposition a "follicular relic" remains, which is seen as a dilatation on the pedicel of the ovariole (Detinova, 1962:25-29; 1968). Polovodova (1949) was the first to use this as a means of determining parity in mosquitoes (with *Anopheles*), but the method was not generally known to the non-Russian speaking world until a 1958 review by Gillies. The Polovodova technique proved to be superior to the ovarian tracheation method to distinguish parous from nulliparous female *tarsalis* in a laboratory colony and in the field (Nelson, 1966), but Nelson (1964:243) was unable to determine the number of ovipositions with this technique. Rosay (1969:608) commonly found fewer dilatations than ovipositions in *tarsalis* females of known history, and she reported dilatations in specimens that had never oviposited.

DISSECTIONS

In the laboratory, field-collected mosquitoes were immobilized by chloroform, sorted to species and sex, and counted. Each *tarsalis* female was placed in a glass "embedding button" (5 mm deep, 20 mm inside diam) with 2 drops of 0.675% NaCl. The buttons were stacked to retain moisture. Identification of each female was verified under 20X of a Bausch and Lomb stereoscopic dissecting microscope, and the external condition was recorded. The mosquito was then oriented ventral side uppermost and head to the left, and held by the thorax with a pair of fine forceps. A minuten pin dissecting needle was inserted between the cerci, and by slight downward pressure the 3 hard, brown, oval spermathecae and their ducts and surrounding tissue were withdrawn. In most cases, active sperm could be seen without crushing the spermathecae. In doubtful cases the spermathecae were transferred to a slide with a drop of saline solution and examined at 100X with a compound microscope.

The points of a second pair of fine forceps were then inserted into the lateral portions of the membrane between the 6th and 7th abdominal segments, and drawn to the right, freeing the gut and ovaries in most cases. The remaining portion of the abdomen was teased open and the degree of fat body development and the presence or absence of muscle remnants were recorded. The midgut was checked for the presence of the meconium and for the presence and degree of digestion of blood.

After the ovaries were separated, 1 was placed on a microscope slide marked with a grid to facilitate counting of developing ova. With minuten pins, the ovarian sheath was removed, and the ovarioles separated. A record was made of Christophers' stage of ovarian development and the presence or absence of dilatations on the pedicel. Two rows of 5 circles were marked on a microscope slide with a grease pencil. If the oocytes were not obscured by yolk (Stages I and II), the second ovary was placed in a drop of distilled water in one of the circles on the slide. When the ovaries had dried, the presence or absence of skeins could be determined at a magnification of 100X.

SOURCES OF ERROR

Generally, for each blood meal taken by a female mosquito a complement of eggs develops, and eggs will not develop without a blood meal. A gravid or parous female is therefore considered to have a history of blood feeding, and a nulliparous female cannot have taken any blood. However, females of some populations of mosquitoes do not always develop eggs after a blood meal (gonotrophic dissociation, gonotrophic discordance) and others develop eggs without blood (autogeny).

Gonotrophic dissociation

"Gonotrophic dissociation" (Swellengrebel, 1929:1378) occurs in several species of *Anopheles* when fat body development rather than egg maturation follows a blood meal in overwintering females (Guelmino, 1951). In the same genus, some species are known to require more than 1 blood meal to initiate egg development. Rao (1947:44) called this phenomenon "gonotrophic discordance". Females that have blood-fed but have not developed eggs are "pre-gravids" (Gillies, 1954:59). Eldridge (1968) has reported that low temperature stimulates gonotrophic dissociation in *Culex pipiens* and suggests that it may occur in *tarsalis* as well, but it has not been observed in several studies (e.g. Bellamy and Reeves, 1963:322).

Autogeny

The adjective "autogene" was coined by Roubaud (1929) to describe females of a population of *Culex pipiens* that produced a small raft of eggs without feeding. "Autogeny" is currently used for the phenomenon of the development of the ovarioles beyond the resting stage without a blood meal (Spielman, 1957:404). The source for ovarian development comes from reserves accumulated during the larval stage with the result that only 1 complement of eggs is produced autogenously. Autogeny in a colony of *tarsalis* was first reported by Bellamy and Kardos (1958) and by Chao (1958) in a subcolony of the same San Joaquin Valley strain. Moore (1963) found high levels of autogeny in field populations in Sacramento Valley, California.

If the percentage of gravid or parous females is to be used as an estimate of blood feeding, this estimate will be too high if the population is autogenous. The complications in the interpretation of data on gonoactivity and parity in *tarsalis* due to autogeny have been discussed by various workers (Burdick and Kardos, 1963:528; Kardos and Bellamy, 1961:450; Kliewer et al., 1969:17; Nelson, 1966: 11). In order to determine the degree of error produced by autogeny, I attempted to estimate its extent in the Wister populations. The following experiments and observations were of a preliminary nature. Only those aspects of autogeny were studied that related to physiological age-grading.

Autogeny is a somewhat elusive phenomenon to investigate because of the apparent interplay among hereditary and environmental factors. It is genetically determined (Spielman, 1957), but its expression in *tarsalis* depends on larval nutrition, photoperiod and temperature (Chaniotis, 1960; Harwood, 1967; Kardos, 1959). Therefore, these 3 factors were kept constant for all laboratory colonies.

Several autogenous colonies of *tarsalis* were established from immatures and adults collected from Wister and vicinity. When workers first attempted to colonize *tarsalis*, they were successful only after subjecting the mosquitoes to a simulated twilight in a large cage (Brennan and Harwood, 1953). I was able to colonize the mosquitoes from Wister in a 1 ft^3 (0.028 m^3) cage without simulated twilight. A photoperiod of 14 hr. light and 10 hr. dark was maintained. Temperature in the colony room fluctuated between 24° C and 27° C and relative humidity varied from 53% to 58%. To increase humidity in the cages, I covered them with plastic bags. Moist raisins were provided as a carbohydrate source. Larvae, reared in 2-liter white enamel pans, were fed either Fleischmann's yeast or laboratory chow (Purina Pigeon Checkers) but this diet was not standardized by weight. The water was aerated to prevent the formation of surface scum.

Three separate criteria were used for detecting autogeny: (1) development of eggs without a blood meal, (2) oviposition without a blood meal, (3) occurrence of Stage III eggs in nonblooded females. Percentage of females that develop eggs without a blood meal was the best index of autogeny. Females with Stage III-V eggs 5-10 days after emergence from pupae were considered autogenous. The second index, percentage of females ovipositing without a blood meal, was used as

Table 1. – Autogeny in *Culex tarsalis* collected as larvae and pupae at Wister and vicinity and reared in the laboratory. 1969.

			NON-BLOOD-FED						**BLOOD-FED**		
			Index of Autogeny			*Mean No. Eggs per ♀*			*Mean No. Eggs per ♀*		
month collected	site	generation	percent gravid	percent ovipositing	N	developing	oviposited	N	developing	oviposited	N
May	14	F_1		24	436		(162)	3			
Jun	11	P					83	11			
		F_1		40	55						
		F_5							202	153	18
Jul	8	P		5	2057		57	14			
Sep	11	-	47		32	119		10			
	8	-	70		23	105		10			
Oct	11	-	17		30	(42)		5			
	8	-	76		49	77		31			
	15	-	9		33	(66)		3			
	8,11,15	-	83	74	23	96	96	19	212	159	16
Nov	11	-	0		12						
	8	-	55		42	96		18			

a measure of autogeny when females were not dissected. This index tended to be lower than the first, because some females developed eggs but did not oviposit. Also, the number of eggs oviposited tended to be lower, because some of the eggs were occasionally retained in the ovaries. The third index of autogeny, percentage of Stage III females without blood in the midgut, was used on field-collected females. In the laboratory, females with anautogenously developed ovaries always had blood in the midgut until after Stage III. Therefore, nonblooded females from the field with Stage III ovaries were classified as autogenous. This index was lower than the first 2 because many females that oviposit autogenously subsequently blood feed and develop eggs anautogenously.

In June 1969, I found several egg rafts in a colony of non-blood-fed mosquitoes that had been reared from larvae collected at site 12. This colony was maintained for 6 generations without a blood meal before I discontinued it 5 months later. (Each new generation was transferred to a separate cage.) The colony from site 14 was allowed to feed during the parent generation, but thereafter it reproduced autogenously for 6 generations (6 months) before it was discontinued. The colony from site 8 died after 12 generations (10 months) without a blood meal.

Tables 1 and 2 show the preliminary data from experiments and field observations of autogeny: Table 1 for females that were collected as larvae or pupae in the field and table 2 for adult collections. For colonies, the generation (P, F_1, F_2, etc.) is designated. For each collection, the percentage of autogenous females is shown beneath the index used for its determination. Data based on 〈10 observa-

Table 2. – Autogeny in *Culex tarsalis* collected as adults in CO_2 traps and shelters at Wister. 1969.

			NON-BLOOD-FED							BLOOD-FED		
			Index of Autogeny				*Mean No. Eggs per ♀*			*Mean No. Eggs per ♀*		
month collected	site	generation	percent gravid	percent ovipositing	percent Stage III	N	developing	oviposited	N	developing	oviposited	N
CO_2 trap												
Jun	2	P						20	11			
		F_1		13		76						
		-	12			228	22		25			
Jul		-	20			61	22		11			
Sep		-	20			25	(27)		4			
Oct		-	22			41						
Shelter												
All*		-			30	155	58		10	(113)		9
Jan (1968)	5	-									138	11

* Females collected from sites 1,2,5-7. September 1967-April 1970.

tions are bracketed. Two site numbers are given that are not shown in the map of Wister (fig. 2): Site 14 was a grassy roadside ditch (ca. 60 cm x 300 cm x 12 cm deep), ca. 8 km south of Wister on State Highway 111. Site 15 was a foul, grassy ditch (ca. 1 m x 2 m x 0.5 m deep), ca. 40 km south of Wister on State Highway 86 at the Keystone Avenue intersection.

Consistently, fewer eggs per female were developed autogenously than anautogenously. From a combined collection from sites 8, 11 and 15 in October, 16 of 39 females were allowed to feed on a pigeon, and all females were isolated in 9-dram (16 g) plastic vials. The bottom 1 cm of each vial was filled with water, and a moist raisin was placed on the cheesecloth top. Eighty-three percent of the non-blood-fed individuals developed eggs autogenously, and 74% of them oviposited. The mean number of eggs that developed for 19 females was 96 as opposed to 212 for the blood-fed individuals. The difference between the means was significant ($p = \langle 0.001$). However, the range overlapped: As many as 136 eggs developed autogenously in 1 female and as few as 118 developed anautogenously. In other colonies, the number of eggs oviposited autogenously ranged up to 186 (site 14, F_1), and 1 blood-fed female developed only 25 eggs (site 11, F_5). Females that took small blood meals usually developed fewer eggs. In the Sacramento Valley, Moore (1963:240) reported that 1 female developed 220 eggs autogenously. Mean number of autogenously developing eggs was highest in May at 116 and declined to less than 35 in October. In June, 92% of the females collected as pupae were autogenous. By October only about 20% were autogenous. Burdick and Kardos (1963:533), using intermediate ovarian tracheation as the criterion, concluded that autogeny decreased from September to October in the San Joaquin Valley.

In August and September 1968, 4 gravid females were collected in CO_2 traps at Wister. In 1969, about 20% of the females caught in CO_2 traps from June to October were gravid (table 2). With 1 exception, no engorged females were collected in these traps. None of the females with Stage III ovaries had any trace of blood in the midgut. Therefore, all of the gravid females in CO_2 traps were probably developing eggs autogenously. The average number of developing eggs per female was 22. This is much less than the number of eggs normally produced by autogenous females. The non-blood-fed females of 2 filial generations developed approximately the normal number of eggs for autogenous females. It may be that the field individuals had not stored sufficient reserves during the larval stage to complete the development of a normal number of eggs autogenously. Ovarian development began, but when reserves were exhausted an additional source of protein was sought in the form of a blood meal. An alternative explanation is that these were females that had taken a very small blood meal and had begun ovarian development. By Stage III, the blood meal was completely digested, and they were attracted to CO_2 in search of a second blood meal. In either case, the gravid females in CO_2 traps did not need a blood meal to complete development of their eggs and to oviposit. In 11 such females, an average of 20 eggs was deposited, of which most hatched.

Two criteria for distinguishing parous from nulliparous females are less accurate when the population is autogenous: ovarian tracheation and follicular relics. The degree of stretch of the tracheal skeins is determined by the number of eggs in the ovaries. The skeins of autogenous-parous females are intermediate between those of nulliparous and blooded-parous females, because autogenous females develop fewer eggs. Burdick and Kardos (1963:531) examined a large series of laboratory-reared *tarsalis* females (with histories of ovarian activity unknown to them until after the experiment) in an attempt to separate them into nulliparous, autogenous-parous, and blooded-parous categories on the basis of ovarian tracheation. By assigning all doubtful specimens to the intermediate (autogenous) category, no nulliparous specimens were incorrectly identified as blooded-parous and only 4% of the blooded-parous specimens were judged to be nulliparous. However, so many females in these 2 categories were incorrectly judged to be autogenous-parous, that only 39% of the nulliparous specimens and 68% of the blooded-parous specimens were correctly identified. Eighty percent of the autogenous-parous females were correctly identified. The other 20% were approximately evenly distributed between the other 2 categories, but of all females identified as autogenous-parous, less than a third were correctly identified.

Follicular relics occur in both autogenous-parous and blooded-parous females. Usually more ovarioles of blooded-parous females will have relics because more eggs are developed, but this criterion cannot be used to distinguish them from autogenous-parous females because of the overlap in the range of the number of eggs developed by these 2 groups. Figures 10 and 11 show the frequency distribution of the number of developing eggs per female from collections at Wister. Based on my laboratory observations (table 1), I expected the following 3 peaks in egg frequencies, with a minimum of overlap: retained eggs (ca. 1-10), autogenously developing eggs (ca. 50-100), and anautogenously developing eggs (ca. 200-250), However, there was no clear division into these 3 groups: 116 of the 315 females examined had from 1 to 7 Stage V eggs; 70 of these had 1 egg. These were probably all females with retained eggs, but the Stage V eggs of females with more than 7 may also have been retained. The rest of the distribution was continuous and did not separate into an autogenous and an anautogenous group. Therefore, the frequency distribution of follicular dilatations is not useful to separate autogenous-parous from blood-fed-parous females. However, lack of dilatations is a good criterion for identifying nulliparous individuals (Nelson, 1966).

In summary, at Wister autogenous-gravid or parous females could not be distinguished from blood-fed-gravid or parous females with a high level of accuracy. However, if monthly levels of autogeny in Imperial Valley are similar to those in Central Valley, which probably is the case, based on my limited data and on Central Valley studies, then the bias due to autogeny should be the same in both areas, and the physiological age-grading data can be compared.

ANALYSIS OF DATA

All of the data from each of the 2658 dissections (date, dissection number, collecting method, site and all the age-grading results) were coded and entered onto standard IBM punch cards. The following programs were written in Fortran IV for a Watfor compiler (Blatt, 1968) and were executed on the model IBM 360/91 of the Campus Computing Network of the University of California, Los Angeles: (1) a print-out of all or any portion of the dissection data in easily readable, non-coded form, (2) a 2-way classification table of a variable number of categories to show the frequency of females for each combination of categories (e.g. if the 2 variables were *external wear*, "ext", and *fat body development*, "fat", the table showed frequency of females with the combination of categories ext = 1, fat = 0; ext = 1, fat = 1; ext = 2, fat = 1; etc. for all possible combinations), (3) percentage of females in each of the 6 stages of ovarian development, tabulated by collection method and by month, (4) percentage of females inseminated each month, tabulated by Christopher's stage and by collecting method, (5) histogram of the frequency distribution of the number of developing eggs per female for all months.

Other computations were executed by an Olivetti Underwood Programma 101 desk computer. A prewritten program (Williams, 1968:33) was used to calculate the monthly mean number of males and females per collecting method and the standard error of the mean. A t-test (Williams, 1968:188) was employed to detect the degree of difference between the mean number of eggs developed by blood-fed vs. non-blood-fed females. Short programs were written for computing percentages and confidence intervals.

RESULTS AND DISCUSSION

WEATHER

A standard U.S. Weather Bureau Station was established at Wister in May 1967 but was discontinued in May 1968. The closest other station with complete weather data was operated by the Imperial Irrigation District, about 50 km south of Wister. Differences in temperature were minimal between the 2 stations for the 13 months in question. The monthly mean lows averaged less than 1° C lower at Wister, and the mean highs less than 0.5° C lower. Recorded rainfall was also nearly the same. Therefore weather data for my entire study were taken from the Imperial station.

Winters are mild and summers very hot at Imperial, California (fig. 12, table 3). The coldest months were December and January, but temperatures only rarely dropped below 0° C: not at all in winter 1967-1968, 4 days in December 1968 and 6 days in January 1970. The lowest temperature during the study was –2.8° C for 1 night in December 1968. The mean low temperature for the coldest month was about 5.3° C for 2 winters and 3.3° C for the winter 1968-1969. Summer temperature reached 47.2° C in August 1970. Mean maximum temperature for the hottest month (July or August) exceeded 40° C all 3 summers. Rain occurred sporadically and infrequently, usually in the winter except for a major storm in July 1968. Average annual rainfall for all years recorded by the Imperial station

Table 3. – Winter and summer temperatures (°C) at Imperial, California.

		1967		1968		1969		1970
WINTER								
Coldest month	: mean		11.7		11.1		12.8	
	mean minimum		5.0		3.3		5.6	
Coldest 2 weeks	: mean minimum		3.9		2.2		2.8	
Coldest day	: minimum		0.6		-2.8		-1.7	
SUMMER								
Hottest month	: mean	34.4		32.8		35.6		
	mean maximum	41.7		40.6		42.8		
Hottest day	: maximum	45.0		45.6		47.2		

was 72.7 mm. Relative humidity, recorded daily at 7:30 a.m. PST at Wister from May 1967 to May 1968, ranged from 9% to 98%. Monthly mean relative humidity was approximately 70% in the winter and 50% the rest of the year.

In most areas outside of California, where the winter biology of *tarsalis* has been studied, winters are severe. In southern Alberta, Canada, Shemanchuk (1965: 461) reported an extreme of more than 35° C below zero, and the air temperature was well below freezing nearly every day from November through March. In a western Nebraska study, ambient temperature reached a low of –30.6° C (Keener, 1952:209). In North Dakota, temperatures below –17° C persist for 2 weeks or more, but in central Washington temperatures below –17° C last for only short periods (Anderson and Harwood, 1966:6). Rush, Brennan and Eklund (1958:291-292) reported below freezing temperatures all during January.

In the San Joaquin Valley, California, winter temperatures are milder, but still considerably colder than the irrigated desert of Imperial County, only 2 degrees of latitude to the south. In Kern County, Bellamy and Reeves (1963:318) reported average minimum temperatures of well below 5° C for many 2-week periods during the winter, and there were biweekly lows below –1° C both winters of Nelson's (1964:249) study. Washino and Bellamy (1963:162) reported that temperature fell below freezing and reported a minimum of –7.8° C for 2 days in January. In Fresno County, Kliewer (1969) showed minimum temperatures below freezing for nearly every week during a 2-4 month period for each of 5 years.

POPULATION DENSITY

The numbers of *tarsalis* adults collected by the 3 methods are listed in table 4. Light traps with CO_2 attractant, used only during the first 11 collecting periods (lunar months) of the study, attracted by far the greatest number of adults per trap per night (trap-night), and no trap failed to capture at least a few adults, except in occasional cases of mechanical failure. CO_2 traps and shelters yielded fewer adults per collection, or sometimes none.

Table 4. – Number of adult *Culex tarsalis* collected by 3 methods at Wister, California. September 1967 – April 1970.

	Number Collected	Number of Collecting Periods	Number of Collections	Mean Number per Collection
Light trap with CO_2	33235	11	280	119
CO_2 trap	6115	33	444	14
Shelter	5171	30	366	14
Total	44521			

MOBILITY

If adults are not active every day, or if the population is very small, progressively fewer mosquitoes will be collected at the same site on consecutive days. At Wister, no effect of the 3 sampling procedures on the population was observed. Collecting from a particular site one day tended neither to decrease nor increase the number of mosquitoes collected the next day. Of 134 such comparisons with light traps, 56% were decreases and 44% were increases. (Instances of no change from one day to the next were not counted.) For CO_2 trap collections, 46% of 211 comparisons were decreases, and of 314 comparisons for shelter collections, 44% were decreases. For all 3 methods, decreases and increases for consecutive collections were about equal, even during the winter months when the population density was low. It may be inferred that the population of adults was very large in comparison to the numbers removed by the 3 sampling techniques, and adults were very mobile all year.

In the northwestern United States and Canada, overwintering *tarsalis* females are nearly completely immobilized by the cold (Rush et al., 1958:291); Shemanchuk, 1965:459). Rush (1962:179-180) collected wintering *tarsalis* females in the field and subjected them to various temperatures in the laboratory. Although there was some survival at temperatures as low as –10° C for 24 hours, at –3.3° C only very slow walking was observed, and true flight was not possible until 2.2° C. Strong flight of 9 meters or more was not achieved until 4.4° C. In the San Joaquin Valley, California, where winter temperatures are less severe, overwintering females are immobilized only during brief cold periods, and shelters become repopulated a week after all mosquitoes are collected (Bellamy and Reeves, 1963). In the Imperial Valley, shelters were repopulated daily, and afternoon temperature was never low enough to reduce adult flying ability noticeably.

FEMALES

The best data on the winter fluctuations of the female population density were obtained during the preliminary phase of the project when many samples were taken every month from September 1967 through July 1968 (fig. 13, table 5). The light traps and CO_2 traps showed nearly identical fluctuations, which was to be expected since CO_2 attractant was employed with both. Fewest females per trap-night were found during the late November-early December collecting period, the only time that the number fell below 10 and was less than the number of females of *Culiseta inornata* caught in the same traps. Average monthly minimum temperatures were lowest in December and January during the winter 1967-1968 (fig. 12) and the coldest 2-week period was January 1-15, but progressively more females were obtained by all 3 methods after the collecting period of November 30-December 2. Light traps were not used for the remainder of the study and fewer CO_2 traps were employed. Supplemental shelter collections were made from 3 wooden bridges, but usually only a few adults were found. During the next 2 winters, the fewest females were found after the coldest months. In the winter 1968-1969, the minimum temperature was in December (fig. 12) but the culvert showed the lowest population in February and the CO_2 traps in March (fig. 13, table 6). The trend in 1969-1970 was not clear since collections were discontinued in April. During the coldest months of both winters (December 1968, January 1970), the numbers in the culvert shelter increased while those in CO_2 traps decreased from the previous month.

For both summers, there was an apparent depression in population density during the hottest month, but collection data were incomplete for the first summer, and during both summers there were temporary aberrant weather conditions. In

Table 5. – Population density. Monthly abundance of adult *Culex tarsalis* collected in light traps with CO_2, CO_2 traps, and a culvert shelter (site 6). August 1967–June 1968.

		Light traps with CO_2			CO_2 traps			Culvert				
		♀			♀			♀		♂		
		$\bar{x}$	$\hat{\sigma}_{\bar{x}}$ *	N	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	N	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	N
1967	Sep 27-29	460.9	53.6	21	28.8	8.3	20	-	-	-	-	-
	Nov 2-5	303.3	42.6	28	7.7	1.8	27	-	-	-	-	-
	Nov 30-Dec 2	9.7	3.5	21	0.3	0.3	21	5.7	1.8	21.0	2.1	3
	Dec 28-30	23.5	5.7	21	1.4	0.7	21	11.3	3.2	13.0	4.7	3
1968	Jan 27-30	19.2	4.5	28	1.2	0.4	28	15.0	3.0	2.7	0.9	3
	Feb 29-Mar 2	70.6	13.2	21	8.1	2.4	20	10.7	4.9	12.3	5.8	3
	Mar 25-27	96.6	13.5	21	15.0	4.0	21	19.5	10.5	16.0	-	2
	Apr 27-29	93.9	14.9	21	8.7	2.9	21	19.0	5.7	9.0	2.5	3
	May 25-27	131.1	24.1	21	24.0	5.9	21	10.7	3.2	3.3	1.2	3
	Jun 20-22	39.9	11.3	13	4.4	2.2	7	-	-	-	-	-
	Jul 19-21	9.7	2.4	16	11.0	2.1	3	-	-	-	-	-

* $\hat{\sigma}_{\bar{x}}$ (unbiased estimate of the standard error of the mean) = $\frac{s}{\sqrt{N-1}}$.

Table 6. – Population density. Monthly abundance of adult *Culex tarsalis* collected in CO_2 traps and shelters. September 1968–April 1970.

		Culvert					Bridges					CO_2 traps		
		♀		♂			♀		♂			♀		
		$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	N	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	N	$\bar{x}$	$\hat{\sigma}_{\bar{x}}$	N
1968	Aug 31-Sep 2	164.3	22.4	19.3	3.5	3	4.2	1.2	3.0	1.2	8	105.8	32.9	6
	Sep 22-24	168.3	24.0	104.3	39.0	3	5.2	0.9	4.5	1.1	12	65.8	11.6	9
	Nov 2-4	42.0	18.0	142.3	70.0	3	2.4	0.9	6.7	2.0	12	42.3	12.3	9
	Nov 22-26	14.0	8.5	79.5	41.2	3	2.9	0.9	5.0	1.6	10	5.0	1.5	3
	Dec 11-14	66.3	5.6	101.3	25.1	3	3.3	1.1	5.3	1.5	9	1.2	0.5	6
1969	Jan 18-20	18.3	6.2	7.7	5.8	3	2.2	1.1	0.8	0.3	9	1.7	1.2	9
	Feb 15-17	2.3	1.3	1.7	0.3	3	0.8	0.3	0.4	0.3	9	1.8	0.8	6
	Mar 17-19	3.7	1.8	0.7	0.3	3	0.6	0.2	0.4	0.2	9	0.8	0.6	6
	Apr 19-21	8.7	1.8	2.3	1.3	3	0.6	0.4	0	-	9	5.0	1.2	6
	May 18-21	24.7	10.3	4.7	1.4	3	3.4	0.9	0.9	0.4	9	10.0	3.3	6
	Jun 14-16	64.7	12.7	7.0	1.0	3	5.8	1.5	1.8	0.8	8	51.7	19.0	9
	Jul 11-13	69.3	11.6	6.3	0.3	3	1.7	0.5	0	-	9	63.6	26.2	9
	Aug 14-16	1.7	1.2	0	-	3	0.2	1.4	0	-	9	1.8	0.7	9
	Sep 11-14	72.0	4.4	45.7	10.9	3	2.6	0.9	1.6	0.9	9	11.4	4.0	8
	Oct 10-13	19.8	8.1	11.7	2.4	4	1.2	0.5	1.1	0.4	9	13.1	3.7	9
	Nov 7-10	18.0	12.0	24.7	13.8	3	0.7	0.2	0.2	0.1	9	1.4	1.1	9
	Dec 5-8	1.7	0.3	3.0	2.5	3	0.9	0.5	0.6	0.2	9	1.1	0.6	9
1970	Jan 3-5	5.7	0.7	10.3	2.7	3	1.3	0.4	1.9	0.5	9	0.1	-	9
	Feb 6-8	7.7	2.7	2.0	0.6	3	1.2	0.4	0.1	-	9	4.0	1.7	9
	Mar 6-8	0.3	-	0.7	-	3	0.1	-	0	-	9	1.2	0.5	9
	Apr 11-12	2.0	-	0	-	2	0	-	0	-	6	0	-	6

the summer of 1968, light trap collections were greatest in mid-May and progressively diminished until mid-July, but this collecting method was discontinued after that month. Fewer females were caught in CO_2 traps in June than May, but this collection method was not employed in July. By late August, CO_2 traps attracted 4 times as many as the previous May, and 25 times the June catch. In early July, unusually heavy rains in the nearby Chocolate Mountains caused the canals at Wister to overflow, and a large portion of the area was inundated. This may have lowered the population by flooding the adult resting sites and washing larvae into the Salton Sea. The subsequent increase in population may be attributed to the increase in available breeding ponds as the flood waters receded. In the summer of 1969, a considerable drop in numbers in shelters and CO_2 traps was evident in mid-August. Shelter collections a month before and a month after were high. Shortly before the collecting period in August, there had been a wind storm which may have dispersed the mosquitoes, but high winds during other months had not noticeably reduced the population in shelters. (However, light trap and bait can catches were reduced on windy days.)

The annual population curve is bimodal. Mosquitoes were most abundant in the spring and fall, somewhat less abundant for a short time in the summer and least abundant in winter. Work, Vanis and Wallace (1969) continued the CO_2 baited light trap collections at Wister after I stopped them in July 1968, and they too observed spring and fall population peaks in 1968 and 1969.

Since rainfall in the Imperial Valley is insufficient at almost any time of the year to provide breeding sites, breeding is largely dependent on irrigation practices. Wister is primarily a management area for migratory waterfowl, and the duck ponds are flooded from about August until March. Other areas of Imperial Valley are irrigated in the spring and summer, but the pattern of seasonal abundance is about the same. Magy (1955) maintained light traps in Imperial Valley at sites ranging from about 40 to 65 km south of Wister. In 1954, *tarsalis* females were most abundant in October, with a smaller population peak during April and June. Larval collections showed the same bimodal pattern. Chew and Gunstream (1970: 560) found peak populations of *tarsalis* in light traps in spring and fall in the Coachella Valley, at a site about 50 km northwest of Wister. In a survey of several other areas of southern California, including Imperial Valley, only 1 peak was evident, in the late spring. In all the areas studied, this species was present in light traps every month of the year, and it was the only species besides *Culiseta inornata* that occurred through the winter months in any numbers.

C. tarsalis demonstrates latitudinal differences in seasonality in various parts of its range from western Canada to southern Mexico. Similar differences are seen in several other species, notably *Culiseta inornata*, which is a winter species in Imperial Valley (Chew and Gunstream, 1970:558), but is most active in the spring and autumn in the northern United States and in the summer in Canada (Horsfall, 1955:351; Shemanchuk, 1959:908). Probably the difference in the time of optimal temperature is one of the most important factors influencing this seasonality.

In southern Alberta, Canada, light trap catches of *tarsalis* were highest in July, but by September they dropped to zero (Shemanchuk, 1959:908). In North Dakota, *tarsalis* is inactive sometimes for more than 6 months, but the period of inactivity in central Washington is usually less (Anderson and Harwood, 1966:6). In Washington, the number of adults in light traps were low in May and peaked in July (Hammon, Reeves, Brookman and Gjullin, 1942:281), and by late September virtually all surviving females had migrated to their winter resting sites (Rush, Brennan and Eklund, 1958:289). Emergence of the overwintered population began the first half of March (Rush, Brennan and Eklund, 1958:291; Harwood, 1962:29). In northern Colorado, hibernating females were found in mines at the end of August when the numbers of females attracted to CO_2 traps had declined (Blackmore and Dow, 1962:293). The emergence of overwintered females in that state varied from late March to mid-April (Bennington, Blackmore and Sooter, 1958:298). In western Nebraska, hibernating females disappeared from a cellar at the end of April, but no females were caught in light traps until June (Keener, 1952:208).

In the San Joaquin Valley, California, populations also show a midsummer peak and a winter low, but fall populations are depressed later and spring populations increase sooner. Collections in light traps (Hayes et al., 1958:221; Reeves, 1970: 3), CO_2 traps (Hayes et al., 1958:221), and shelters (Hayes et al., 1958:221; Reeves and Hammon, 1962:176-177) were highest in late July and August. Occasional adults were found throughout the winter in light traps (Hayes et al., 1958: 221), but no mosquitoes were attracted to CO_2 traps during December and January and sometimes not until March or late April (Hayes et al., 1958:221; Nelson, 1964:247).

Jenkins (1950:913) noted that most collection records of *tarsalis* east of the Mississippi River are in late fall. Snow and Pickard (1956:147-148) found peak larval abundance in September in the Tennessee Valley, an area where *tarsalis* is an uncommon species.

Eads (1965) states that on the Lower Rio Grande Valley of Texas, *tarsalis* is a winter mosquito. He presents light trap data for 2 years in which no adults were collected from June to September, although this was the period of greatest abundance for many other mosquito species. On close inspection of his data, I believe that a very definite bimodal pattern is evident for both years. Adults are most abundant in November, common in December and January, and then increase again in February of one year and March of the next. The Lower Rio Grande Valley is semitropical, and the winters are even milder than in Imperial Valley. The average January temperature was 15.6° C, as opposed to 12.2° C at Imperial, but the minimum occasionally dropped below freezing. It is difficult to explain the summer disappearance of *tarsalis* from the Lower Rio Grande, since the average July temperature was 28.9° C, compared to 34.4° C in Imperial Valley, and other species of mosquitoes were abundant. Most of the 760 mm of rainfall occurred in the fall and spring, and standing water was usually more abundant in the winter months, but potential breeding sites were plentiful in the summer due to agricultural irrigation.

Figure 14 is a diagrammatic summary of population fluctuations in the major areas that have been discussed above. *C. tarsalis* is a summer mosquito to the north, a fall and spring species in the Imperial Valley, and a winter mosquito in southern Texas (with a mild midwinter depression). The series cannot be explained entirely by differences in temperature regimes, since the Lower Rio Grande Valley summer temperatures are not excessive.

MALES

Although males collected in shelters at Wister were less abundant in winter than during the fall, they never disappeared (fig. 15, tables 5, 6). During the late fall and early winter of both years, males were distinctly more numerous than females, but during the spring and summer months the converse was true.

Throughout most of the range of *tarsalis*, males do not survive the winter, although Harwood (1962:28-29) did collect 1 male in an emergence trap placed over a talus slope from March 11 to April 29, 1961. Because this collection, and others of male *Anopheles freeborni* Aitken, 1939, were made from a north-facing slope, Harwood (1962:30) speculated that the colder, more stable site was most favorable to hibernation of adults by conserving their energy reserves. Usually, the first males seen in the spring are brightly colored members of the first spring brood, a few weeks after females emerge from their wintering sites (Rush and Tempelis, 1967:309). In southern Alberta, Shemanchuk (1965:459) found females in mammal burrows from September to January and April to June, but males were present in September only. Bennington, Sooter and Baer (1958:300) collected females under a bridge in northern Colorado in mid-April, but males did not appear until late May. Both sexes had virtually disappeared by the end of October. Keener (1952:208) collected females from cellars in western Nebraska from mid-January until the end of April, but no males were found. In Fresno county, California, males were absent from culverts in January, February and March (Kliewer et al., 1969:17), and in Kern County they are absent from shelters for a month or longer, and do not reappear in numbers until late March or April (Bellamy and Reeves, 1963:317; Burdick and Kardos, 1963:533; Nelson, 1964:248).

At Wister, the presence of *tarsalis* males throughout the year is the clearest evidence of a basic difference in winter biology between that population and those mentioned above. Since blooded and gravid females and larvae are also present all winter, there is no distinct "new generation" of adults indicated by the first appearance of males in the spring. Continuous breeding produces a continuous population of males.

The apparent increase in percentage of males in shelters for both winters at Wister may be due to a change in behavior of the females. Perhaps the majority of the females disperse from Wister during the cooler months (p. 12) or seek more secluded resting sites such as animal burrows (p. 10). Bellamy and Reeves (1963: 317,318,321) found a secondary peak of abundance of males in late autumn in Kern County. They thought that there may be an increased tendency for males to congregate in shelters at that season or survival may be better at the cooler autumn temperatures.

IMMATURES

Larvae and pupae were found every month of the year at Wister. Although much of the area was flooded to provide habitat for migratory water fowl, larvae were seldom found in the duck ponds (fig. 6). *Gambusia affinis* (mosquito fish), an efficient predator of mosquito larvae (Hoy and Reed, 1970), was very abundant in the ponds. The best *tarsalis* breeding sites were shallow, grassy ditches (fig. 7) with recent accumulation of seepage from the ponds or the canals. Sporadic breeding occurred at or near almost every site enumerated on fig. 2, especially sites 1, 6 and 12. Unfortunately, these sites were usually dry after a few weeks, and therefore breeding levels over time could not be determined for any one seepage ditch.

Table 7 shows fall and winter collections from site 8, the large sewage oxidation pond which was the only breeding place that contained water all year. One dip was made for each 0.1 m^2 of surface area collected, therefore mean number of larvae per dip can also be interpreted as mean number per 0.1 m^2 of water surface. Lesser numbers of larvae were collected in the winter months, but no trend is readily apparent from the limited data. Frequent changes in the level of

Table 7. – Immatures. Monthly collections of larvae and pupae of *Culex tarsalis* in the sewage oxidation pond (site 8) at Wister, California.

Date of collection		Mean number of immatures per dip	Number of dips
1969	Sep 13	0.8*	60
	Oct 10	50.0*	60
	Nov 9	5.4	60
	Dec 7	9.5	63
1970	Jan 5	0.1^{+}	20
	Feb 7	0.2	20
	Mar 7	0.8	20

* Estimate.

+ Larvae were plentiful in 3 seepage ditches in January.

the pond corresponded to decreases in numbers collected, even from one day to the next. On January 5, 1970, although plentiful larvae of both *Culiseta inornata* and *Culex pipiens quinquefasciatus* were found in the oxidation pond, only 1 larva of *tarsalis* was collected in 20 dips. However, 3 other temporary breeding sites in the area had larger number of *tarsalis* larvae that month.

In the northwestern United States, *tarsalis* larvae are not found in the winter (Rush, Kennedy and Eklund, 1963a) because females are gonoinactive. In Kern County, California, immature stages do not disappear completely in the winter, but they are apparently much less abundant than in Imperial County. Brookman (1950:85) and Washino and Bellamy (1963) found at least a few immature stages of *tarsalis* throughout the winter months, but as the latter authors state, "during the cold months an exhaustive search was necessary to find any larvae." In January, only third and fourth instar larvae and pupae were found, and in February, in 34 potential breeding sites, 7 larvae were found, all of which were first and and second instar. By March, all larval instars were found, but no pupae. Probably, the January population represented the last of the developing immatures from adults that had been gonoactive in the fall, and the February population of early instars was from females that had overwintered and subsequently became gonoactive. In my study at Wister, immatures were more numerous, and pupae and all larval instars were found every winter month.

On January 6, 1970, at 7:00 a.m., I noticed that Wister site 1, a small grassy seepage ditch approximately 0.5 m x 2 m x 10 cm deep, was frozen over. Ambient minimum temperature for the day was –1° C. Under about 2 or 3 cm of ice, 174 *tarsalis* and 207 *Culiseta inornata* pupae and larvae of all instars were collected by pipette. In the laboratory the collection was divided equally between 2 plastic half-liter cups. The first cup was held at 25.6° C and the other was refrigerated. After 16 hours at 0° C, 5 *tarsalis* and 15 *inornata* larvae were immobilized in the ice on the top two-thirds of the cup. The frozen and liquid portions were separated and incubated at 25.6° C with the control. Almost no mortality was observed for any of the 3 groups, and both species continued development to the adult stage.

Washino and Bellamy (1963) found larvae under ice on 2 occasions in Kern

County. Although development is slower and mortality probably higher at winter temperatures, adults emerged from penned pupae in the field.

Immatures of *tarsalis* can also tolerate relatively high temperatures. In June 1968, active larvae were found in a seepage ditch (site 11) about 5 cm deep where the water temperature was 35° C. Jenkins (1950:914) reports that larvae have been found in thermal waters at temperatures up to 39.0° C.

PHYSIOLOGICAL PARAMETERS

FAT BODY DEVELOPMENT

The percentage of females at Wister with Class 3 fat body (abdomen filled with fat but not distended) was highest in November of both years (fig. 16, table 8). Almost no females had abdomens that were noticeably distended by fat (Class 4). No females collected in March, April or May 1969 had more than a trace of fat body in the abdomen (Class 2). The pattern was nearly identical in the CO_2 traps (although the data are less reliable because fewer females were examined). Females with Class 3 fat body were attracted to CO_2 in the same proportion that they were found in shelters each month. Diapausing females should not be attracted to CO_2 because they do not feed; therefore the maximum amount of fat body developed by females at Wister was not characteristic of diapause.

In other areas where the winter biology of *tarsalis* has been investigated, fat body is strongly developed. Where winters are coldest, *tarsalis* begins to store energy reserves in early fall. By late fall, the abdomen is distended by a compact, lobular fat body (Bennington, Sooter and Baer, 1958:301; Blackmore and Dow, 1962: 293; Harwood and Halfhill, 1964; Rush, Brennan and Eklund, 1958:289). Apparently in the San Joaquin Valley, California, fat body develops less than in Washington (Harwood and Halfhill, 1964:597,598). Burdick and Kardos (1963:533) reported that the largest percentage of females with abdomens filled or distended with fat was in late December (91%) in Kern County. At Wister, the highest level was in November of both years: 59% in 1968 and 38% in 1969.

The differences in degree and duration of fat body development may be due to a direct effect of temperature or to latitudinal differences in response to photoperiod. Although decreasing day length seems to be the important factor for initiation of fat body development in *tarsalis*, its effect is reinforced by low temperature (Harwood and Halfhill, 1964; Harwood and Takata, 1965). Not all populations of the same species of mosquito show the same response to photoperiod, even under a controlled temperature regime. In a laboratory strain of *tarsalis* from Bakersfield, California, Harwood and Halfhill (1964:597) never observed fat body as large or as compact as that found in hibernating females in central Washington. This may reflect a difference in the natural populations of the 2 areas or it may be due to changes in the Bakersfield colony after many years under laboratory conditions. California and Washington strains of *Anopheles freeborni* showed latitudinal differences in fat body response to photoperiod (Depner and Harwood, 1966). The Wister population of *tarsalis* shows no obvious difference in time of initiation of fat body development from the other populations that have been studied, but, probably due to the warmer fall and winter temperatures, the fat body never develops to the same extent, and it is more rapidly metabolized as the females continue to be active.

Table 8. – Fat body development in female *Culex tarsalis* from shelters and CO_2 traps.

DATES	SHELTERS					CO_2 TRAPS				
	Fat Body Class (%)					*Fat Body Class (%)*				
	0	1	2	3	N	0	1	2	3	N
1968 Aug 31-Sep 2			95	5	38	3	16	66	16	77
Sep 22-24	13	1	72	15	95	12	0	54	34	67
Nov 2-4			56	44	32	9	0	41	49	87
Nov 24-26			41	59	29			(33)	(67)	3
Dec 11-14			88	12	101			(43)	(57)	7
1969 Jan 18-20			(71)	(29)	7			(86)	(14)	7
Feb 15-17		58	33	8	12		(50)	(33)	(17)	6
Mar 17-19		(71)	(29)		7		(100)			4
Apr 19-21		52	48		25	4	37	59		27
May 18-21	13	52	35		69	1	57	41		68
Jun 14-16		6	84	10	82	1	1	87	11	171
Jul 11-14			88	12	32			91	9	53
Aug 14-16			(100)		9			82	18	11
Sep 11-14			84	16	74			84	16	25
Oct 10-13			94	6	52			90	10	41
Nov 7-10		2	60	38	42			(38)	(62)	8
Dec 5-8			91	9	11			70	30	10
1970 Jan 3-5					0					0
Feb 6-8			91	9	33			97	3	34

INSEMINATION

C. tarsalis females destined to overwinter usually mate first (Bellamy and Reeves, 1963:319; Burdick and Kardos, 1963:533; Kliewer et al., 1969:15) but insemination is not necessary for the fat body to develop (Harwood and Halfhill, 1964: 598). Since females mate soon after emergence from the pupal stage at all seasons, the high percentage of females inseminated in the winter is probably due to a dearth of immatures at that time that would give rise to noninseminated females.

At Wister, I examined the spermathecae of too few females during some months to form a definite conclusion about the insemination rate in the winter (table 9).

Table 9. – Insemination. Monthly percentage of inseminated female *Culex tarsalis*.

		SHELTER		CO_2 TRAP	
		Percent inseminated	N	Percent inseminated	N
1968	Aug 31-Sep 2	99	92	100	37
	Sep 22-24	86	104	96	57
	Nov 2-4	62	26	94	78
	Nov 24-26	68	44	100	10
	Dec 11-14	84	102	(86)	7
1969	Jan 18-20	98	57	100	13
	Feb 15-17	(100)	4	(100)	1
	Mar 17-19	--	0	--	0
	Apr 19-21	100	19	96	26
	May 18-21	--	0	--	0
	Jun 14-16	--	0	--	0
	Jul 11-13	97	32	98	43
	Aug 14-16	(100)	7	(100)	4
	Sep 11-14	78	63	96	23
	Oct 10-13	82	50	95	38
	Nov 7-10	52	40	(100)	9
	Dec 5-8	46	11	90	10
1970	Jan 3-5	--	0	--	0
	Feb 6-8	97	33	100	34
	Mar 6-8	(100)	1	100	10

In early September 1968, 91 of 92 females examined from shelters were inseminated. The percentage of inseminated females then declined for 2 months to 62% in early November, and then steadily increased to 98% in January 1969. The following winter, there was again a suggestion of a fall decline of insemination rate and subsequent increase by February. Nearly all females captured in CO_2 traps were inseminated.

BLOOD FEEDING AND OVARIAN DEVELOPMENT

Engorged or gravid females were found all year (fig. 17). The percentage of females with blood or developed ovaries was lowest in the late fall, at the same time that percentage of females with fat body was greatest (fig. 16). Before the coldest weeks of winter, fat body development decreased and blood feeding and ovarian development increased. There was another apparent depression of blood feeding and ovarian development in the summer. The data for 1968 are incomplete: the percentage of females engorged or gravid decreased in May and was higher in early September, but shelter collections were not made in June or July. In summer 1969, the lowest level was in July, the month before the apparent depression of the female population (fig. 13). Although August was the hottest month in 1969 (fig. 12), blood feeding increased that month. The level of blood feeding may be influenced by the density of birds at Wister, which is much higher in the winter than in the summer.

Where winters are severe, engorged females are not found for many months. In Utah (Blackmore and Dow, 1962:293) and in Colorado (Bennington, Sooter and Baer, 1958:301), no engorged females are found by October. In the spring, the first females do not bite bait animals until March in Oregon (Rush, Kennedy and Eklund, 1963a:260).

In the San Joaquin Valley, numerous studies have documented the winter depression in blood feeding (Kliewer et al., 1969:15; Nelson, 1964:248; Reeves, Bellamy and Scrivani, 1958:84). Bellamy and Reeves (1963:316,321) found a nearly complete cessation of blood feeding and ovarian development for a period of almost 3 months every year for 4 years, but did not find any period when feeding ceased completely. At Wister, the reduction in blood feeding in the fall was not as extreme as reported for the San Joaquin Valley. In 1967, engorged and gravid females accounted for 23% of the female population during the lowest month of blood feeding (fig. 17), 16% in 1968, and 9% in 1969. Resumption of blood feeding was detected in late December the first two years and in early January of the third. In Kern County, a gradual increase in the percentage of females engorged is usually observed in late January and only rarely as early as late December (Bellamy and Reeves, 1963:316; Nelson, 1964:248).

In the Lower Rio Grande Valley, Texas, where *tarsalis* is essentially a winter species, Eads (1965) collected many engorged or gravid females in light traps from October to January. I have calculated the proportion of engorged or gravid females from the numerical data that he presented: 54% of the females were engorged or gravid in October, but only 19% in November and 34% in December. By January, the level had increased to 63%. No data was given for February or March. These fluctuations in blood feeding occur about a month behind similar fluctuations in population level (p. 26) and, like the population fluctuations, suggest a bimodal curve of activity with a depression in early winter.

The blood meals of 43 females that I collected in September 1969 in the culvert (site 6) were analyzed by precipitin test (Tempelis and Lofy, 1963) by Dr. Tempelis at the University of California, Berkeley (table 10). Of particular interest are the 3 snake bloods. As previously mentioned (p. 5), snakes are currently under study as overwintering candidates of WEE virus. Only rarely are field-collected *tarsalis* females found engorged with reptilian blood. Of over 10,000 engorged females tested, Tempelis et al. (1965:174) detected 3 reptilian bloods in Kern County, and Tempelis and Washino (1967:316) found 1 reptilian blood meal in more than 900 tested in the Sacramento Valley.

Table 10. – Hosts of *Culex tarsalis* as determined by precipitating antisera to blood meals of females collected in shelters. September 1969.

Bird		
	Columbiformes	1
	Negative*	15
Mammal		
	Rabbit	4
	Man	1
Reptile		
	Snake	3
Negative		19

*Class Aves, order not determined.

PARITY

Parous females were found every month of the year at Wister (fig. 18). In shelters, the percentage of parous females decreased in the fall of 1967 and 1969, but by early winter it had increased. This corresponded to the simultaneous increase in blood feeding and ovarian development (fig. 17) and to the decrease in fat body development (fig. 16).

Even at the more northern latitudes, parous females do not disappear completely. In northern Colorado, 2 of 597 females collected in a mine in early December were parous (Blackmore and Dow, 1962:293-294). In eastern Colorado, Rush and Tempelis (1967:311) found parous females in mid-April, before they found evidence of the new spring generation of adults. In the San Joaquin Valley, at least a few parous females are found in shelters throughout the winter (Burdick and Kardos, 1963:532; Kliewer et al., 1969:17). Bellamy and Reeves (1963:319,320) demonstrated experimentally that parous females could survive the winter in Kern County, but survival was inferior to that of females that had not been allowed to take a blood meal.

Although parity of shelter-collected females decreases in the fall in some areas, it increases in females attracted to CO_2. In northern Colorado, progressively fewer females were caught in CO_2 traps in the late summer, but the percentage of those females that were parous reached 100% by late September. The parity of empty females in shelters dropped to zero during the same period (Blackmore and Dow, 1962:292). Nelson (1964:246) reported the same phenomenon in the San Joaquin Valley later in the year. By the end of October, nearly all females collected in CO_2 traps were parous, but parity in empty shelter-collected females had decreased. He suggested that the fall population of *tarsalis* females is divided into two groups: (1) Females that emerge from pupae in the summer take blood meals and oviposit repeatedly for the duration of their lives. They usually do not survive the winter. These are the host-seeking females with a high level of parity found in CO_2 traps in dwindling numbers in the fall. (2) Females that emerge from pupae in the fall experience diapause. They do not blood feed until after they have overwintered (January in Kern County). Hence, few females found wintering in shelters are parous.

At Wister, too few females from CO_2 traps were examined to detect a divergence in level of parity between females from CO_2 traps and those from shelters.

The indication is that there is no divergence. Fluctuations in parity level were similar for the 2 methods from June to October 1969. In mid-October, 56% of 25 CO_2 trap females and 54% of 26 shelter-collected females were parous. In Kern County, from the first to the second half of October 1952, parity decreased from 30% to 17% in shelters and increased from 90% to 93% in CO_2 trap collections (Nelson, 1964:247).

CONCLUSIONS

The Wister population in Imperial Valley, California, remains active all year. Winter diapause is so weakly expressed and of such short duration as to be nearly nonexistent. In the fall, blood feeding and parity are somewhat reduced momentarily and a fat body is developed. However, these indications of diapause begin to disappear even before the coldest days of winter. The amount of fat body developed is less than typical for diapause, and females with a fat body are attracted to CO_2. Males, pupae and larvae of all instars are found throughout the winter.

The duration of diapause is different at various latitudes. In Canada and the northwest United States, it may last 6 months. In California it is shorter. Bennington, Sooter and Baer (1958:303) state that in California "true hibernation" does not occur in *tarsalis* but studies by workers in the San Joaquin Valley have shown almost complete cessation of activity for nearly 3 months. Females develop a fat body and cease feeding almost completely; parous females are rare; males disappear and larvae are very scarce. In the Lower Rio Grande Valley, Texas, there is no winter diapause.

In the Imperial Valley, it does not seem that diapause would be necessary for winter survival of *tarsalis*. Although blood feeding decreases in late fall, it resumes before the coldest weeks of winter. Therefore, the temperature during this period does not appear to inactivate the mosquitoes directly. Progressively shortened day length in the fall may initiate diapause in populations of *tarsalis* at all latitudes, and low temperature probably reinforces the response. The temperature in the fall and winter in the various areas studied is probably largely responsible for the differences observed in degree of expression and duration of diapause. Where winters are less severe, the females are more active, and energy reserves are depleted more rapidly. The stimulus for breaking the diapause may be simply the complete or partial exhaustion of the fat body, or as Bellamy and Reeves (1963:321) suggest, accumulation of a critical amount of heat. In either case, the period of diapause would be shorter where fall and winter temperatures are higher.

Potentially, *tarsalis* in the Imperial Valley is a good candidate for overwintering arboviruses. However, without direct evidence of the viruses in the winter, it is not possible to determine the population density and the level of blood feeding necessary to maintain the cycle of transmission. Work, Vanis and Wallace (1969) made 12 isolations of WEE, 10 of SLE and 21 of Turlock virus from *tarsalis* in 1968 and 1969 at Wister, but none of these isolates was from December or January. Just as in Kern County, California, at Wister no virus has been found when the level of blood feeding is lowest. Further investigations of the viruses and their vectors and reservoir hosts will be necessary for a better understanding of their seasonal relationship.

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Volume 5

No. 1	January 28, 1970
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No. 3	February 27, 1970
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No. 6	June 2, 1970
No. 7	August 17, 1970

Volume 6

No. 1	August 3, 1970
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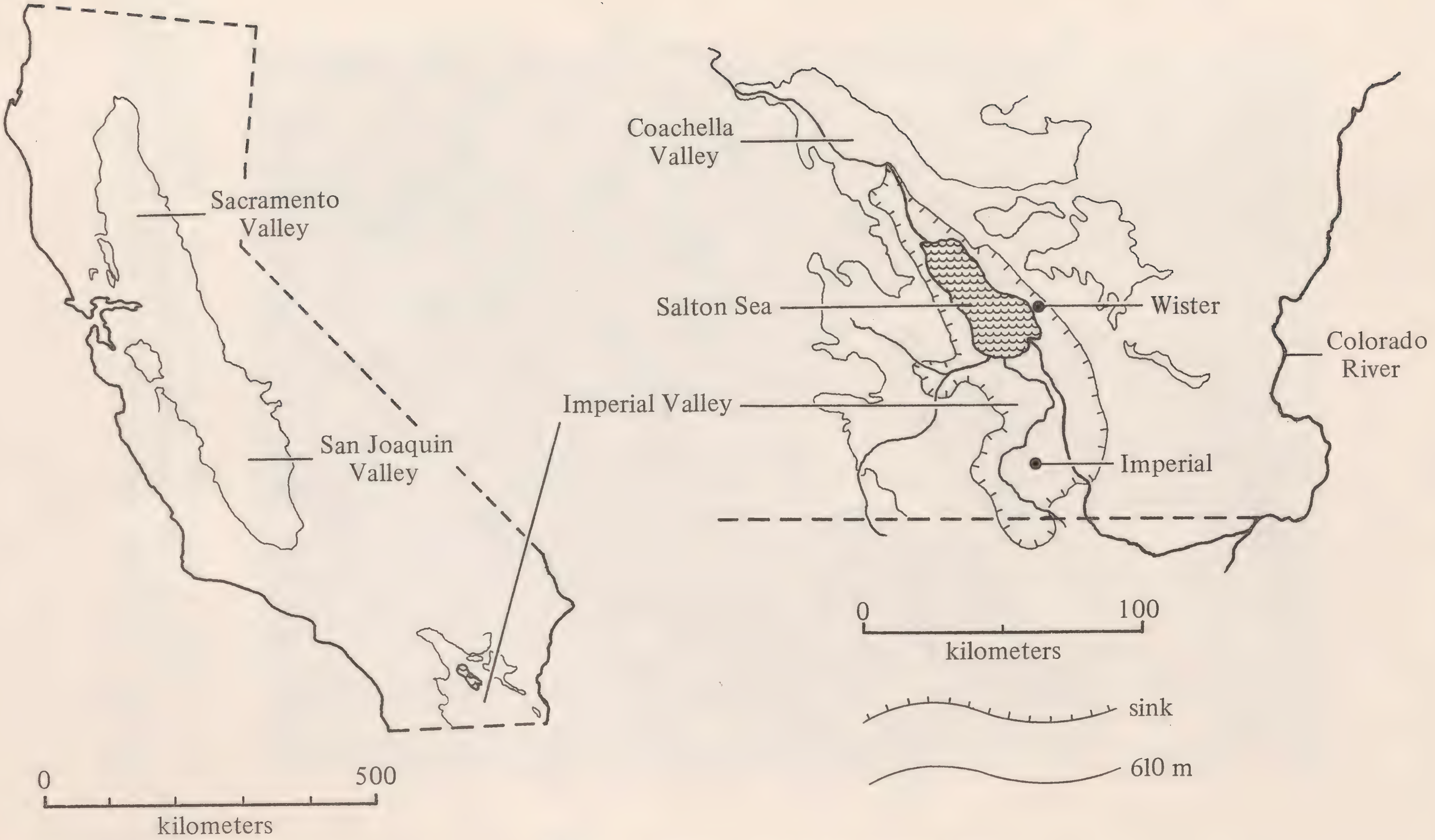

Figure 1.–Maps of California and the Imperial Valley.

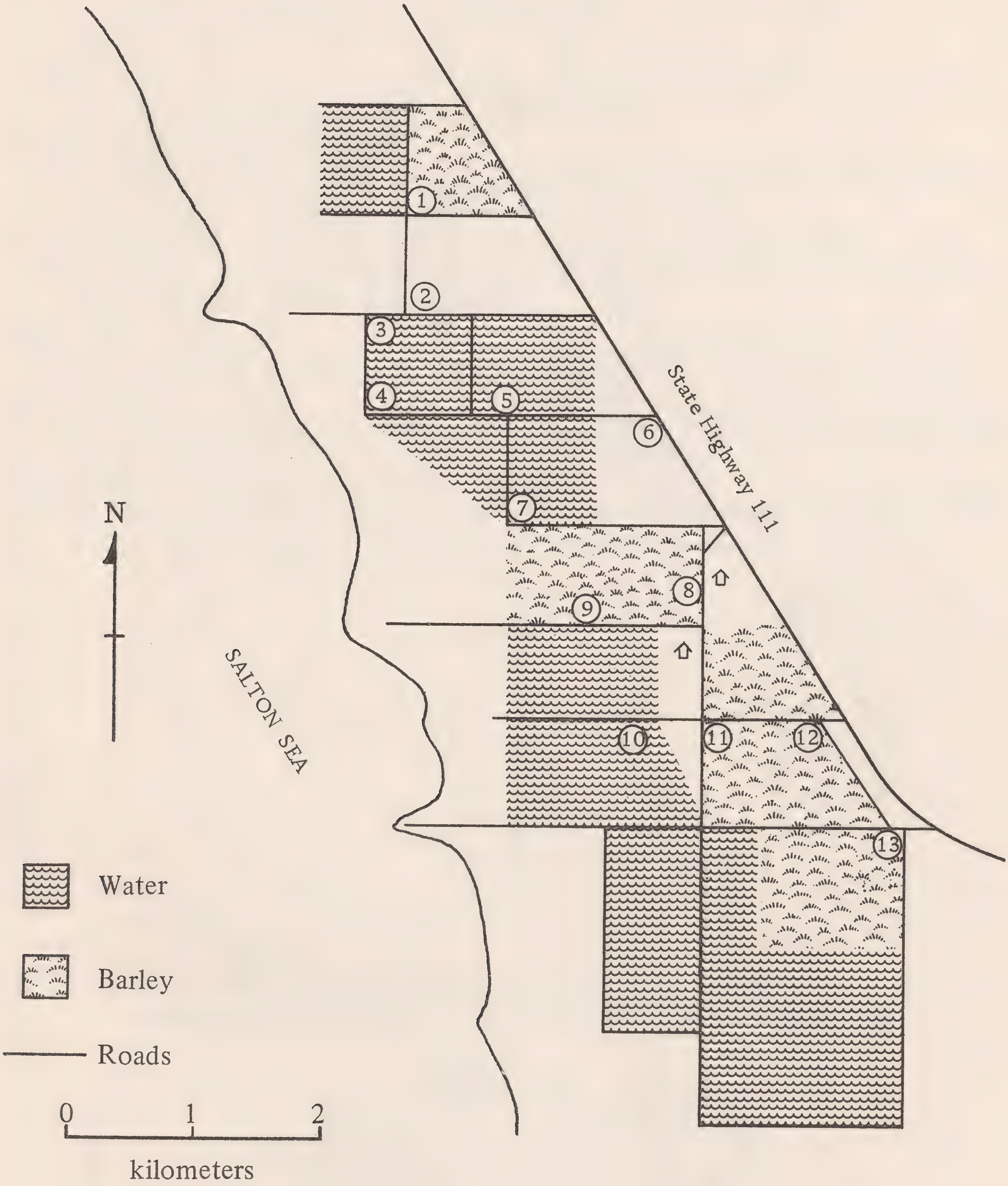

Figure 2.–The Wister Unit of the Imperial Waterfowl Management Area, Imperial County, California. Circled numerals indicate sites where routine collections of *Culex tarsalis* were made.

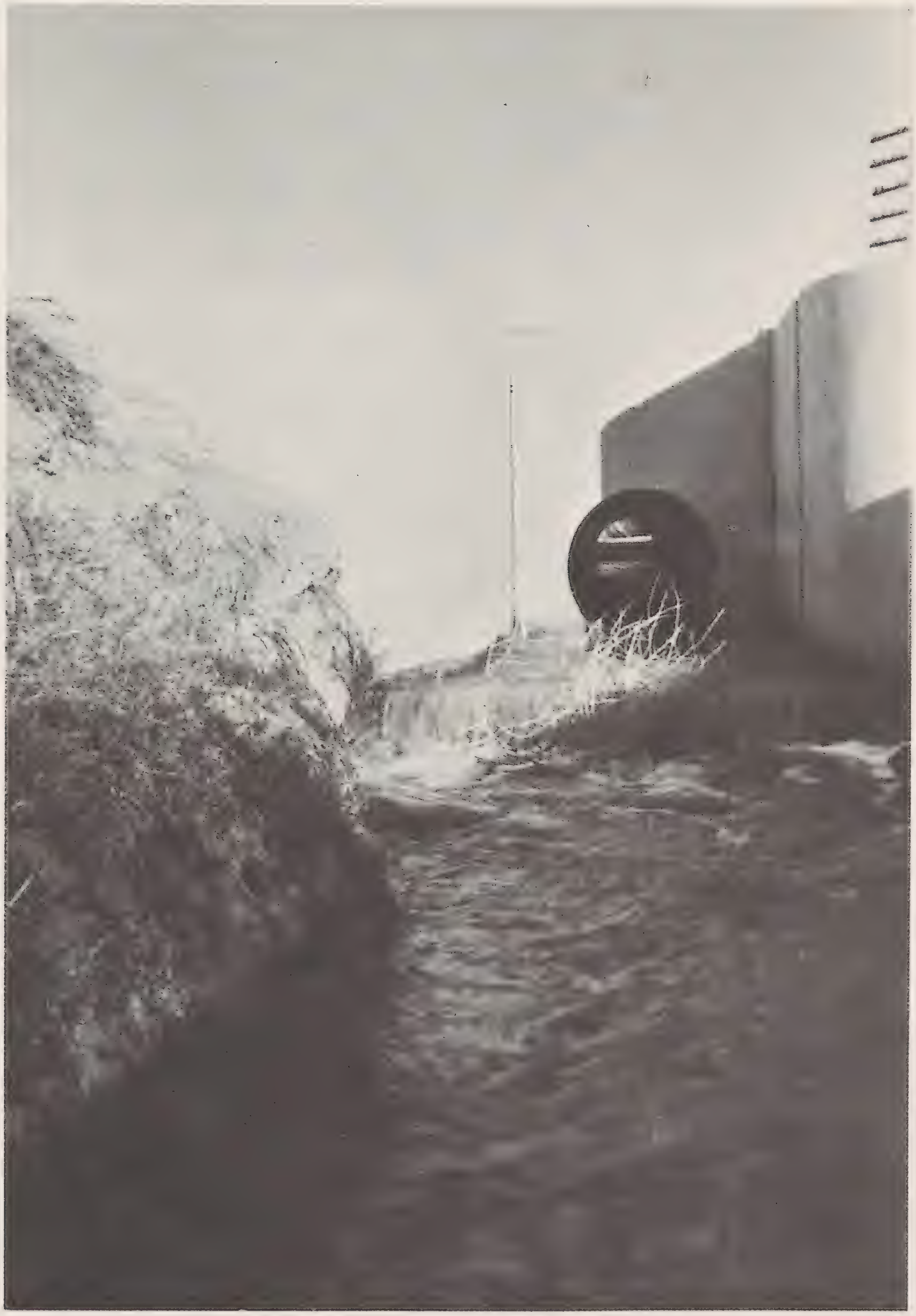

Figure 3.–A diurnal shelter for *Culex tarsalis* adults: a cement culvert (site 6) under California State Highway 111 at Wister.

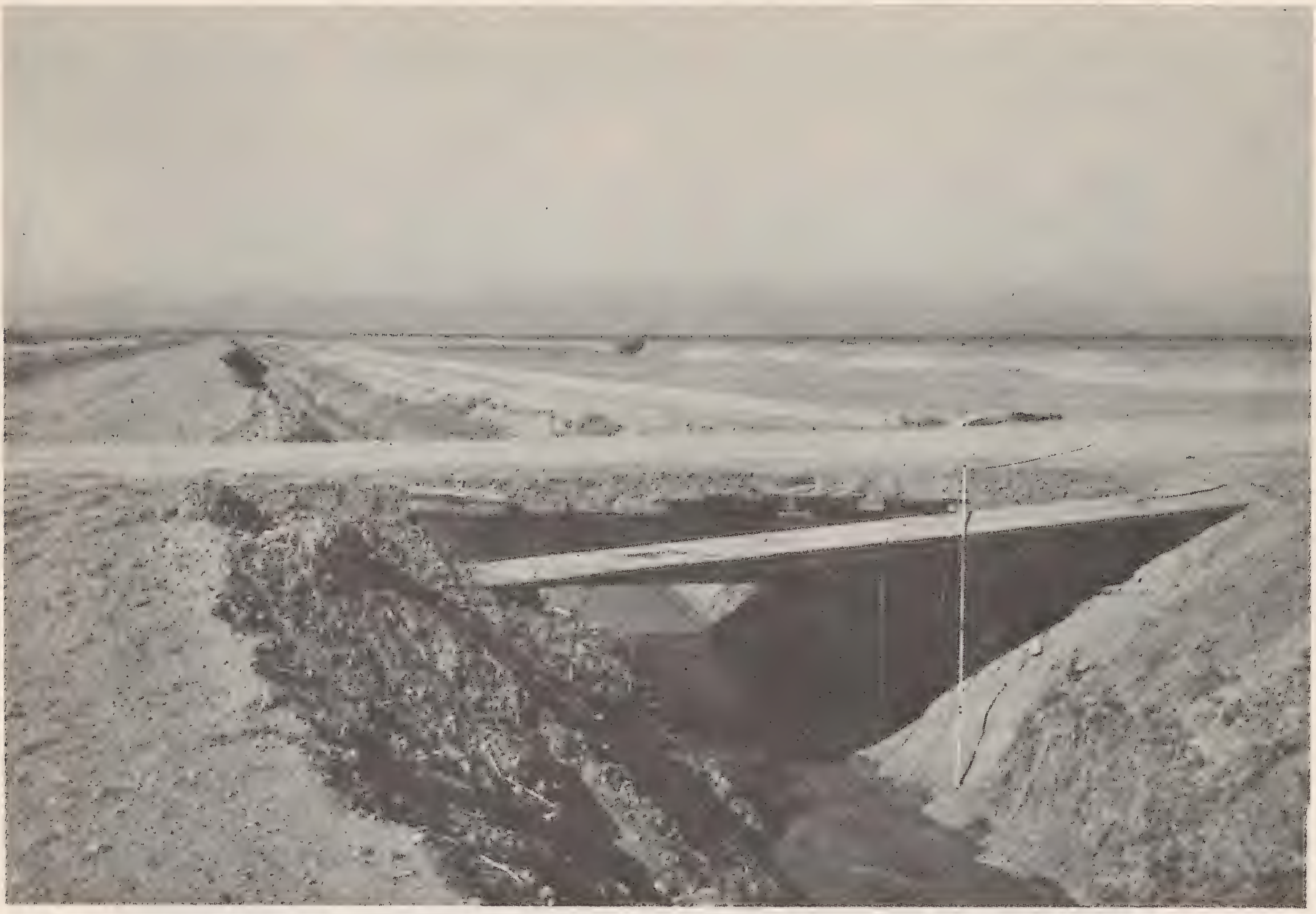

Figure 4.–A diurnal shelter for *Culex tarsalis* adults: a wooden bridge (site 5). The 2 m white rod is marked at 0.5 m intervals.

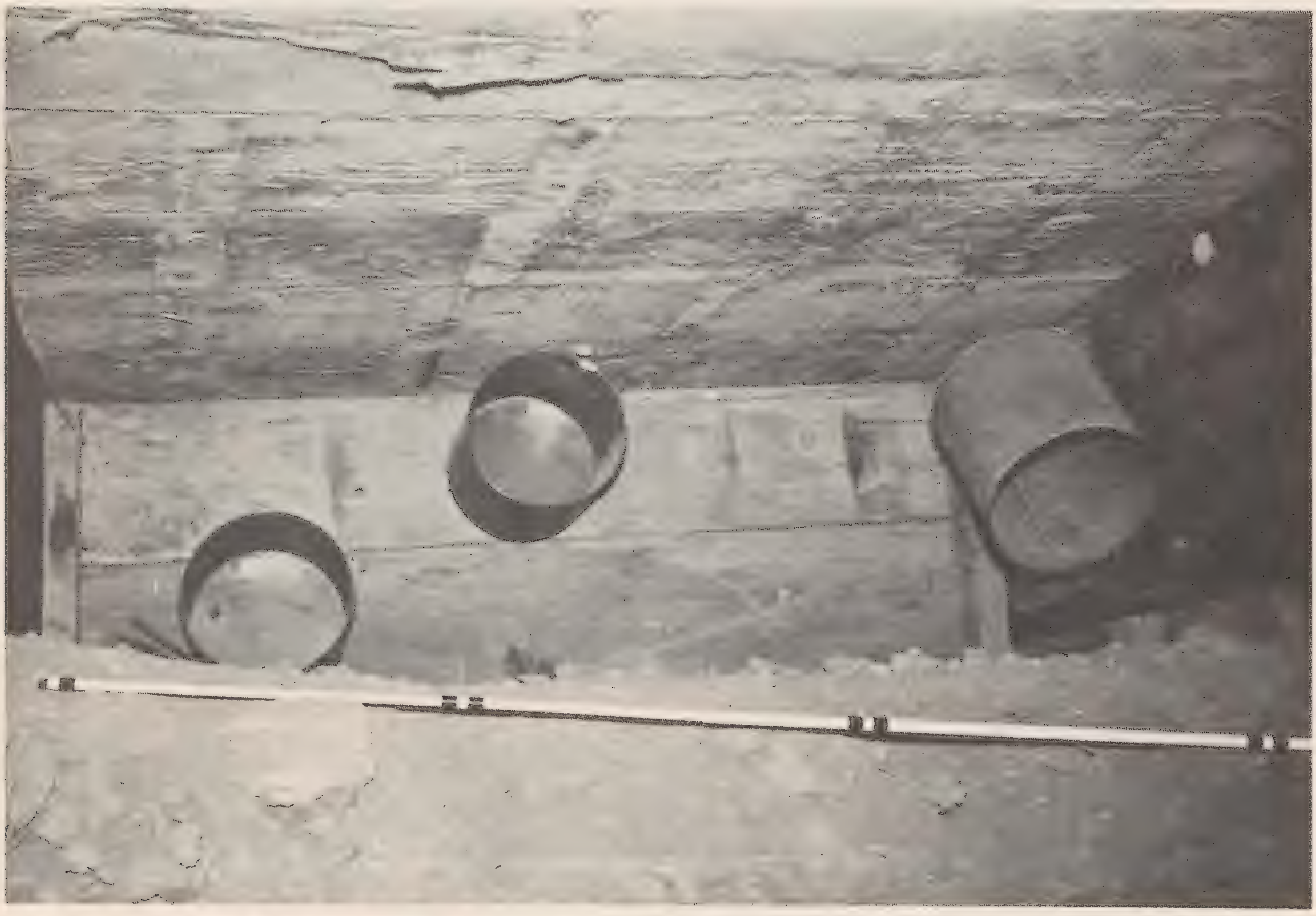

Figure 5.–Red ice cream cartons under a wooden bridge (site 5) from which *Culex tarsalis* adults were collected each month with an aspirator.

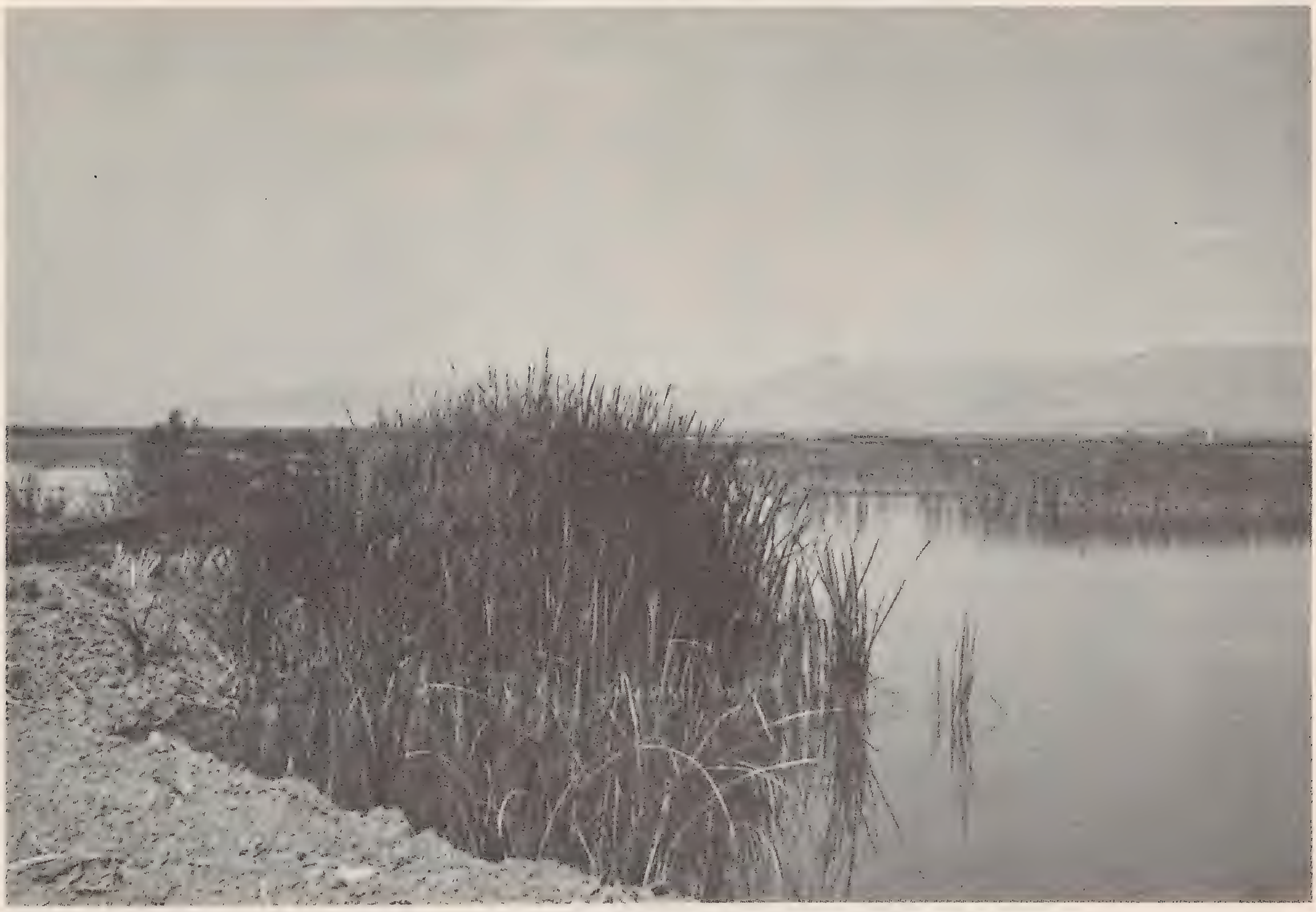

Figure 6.—A duck pond at Wister.

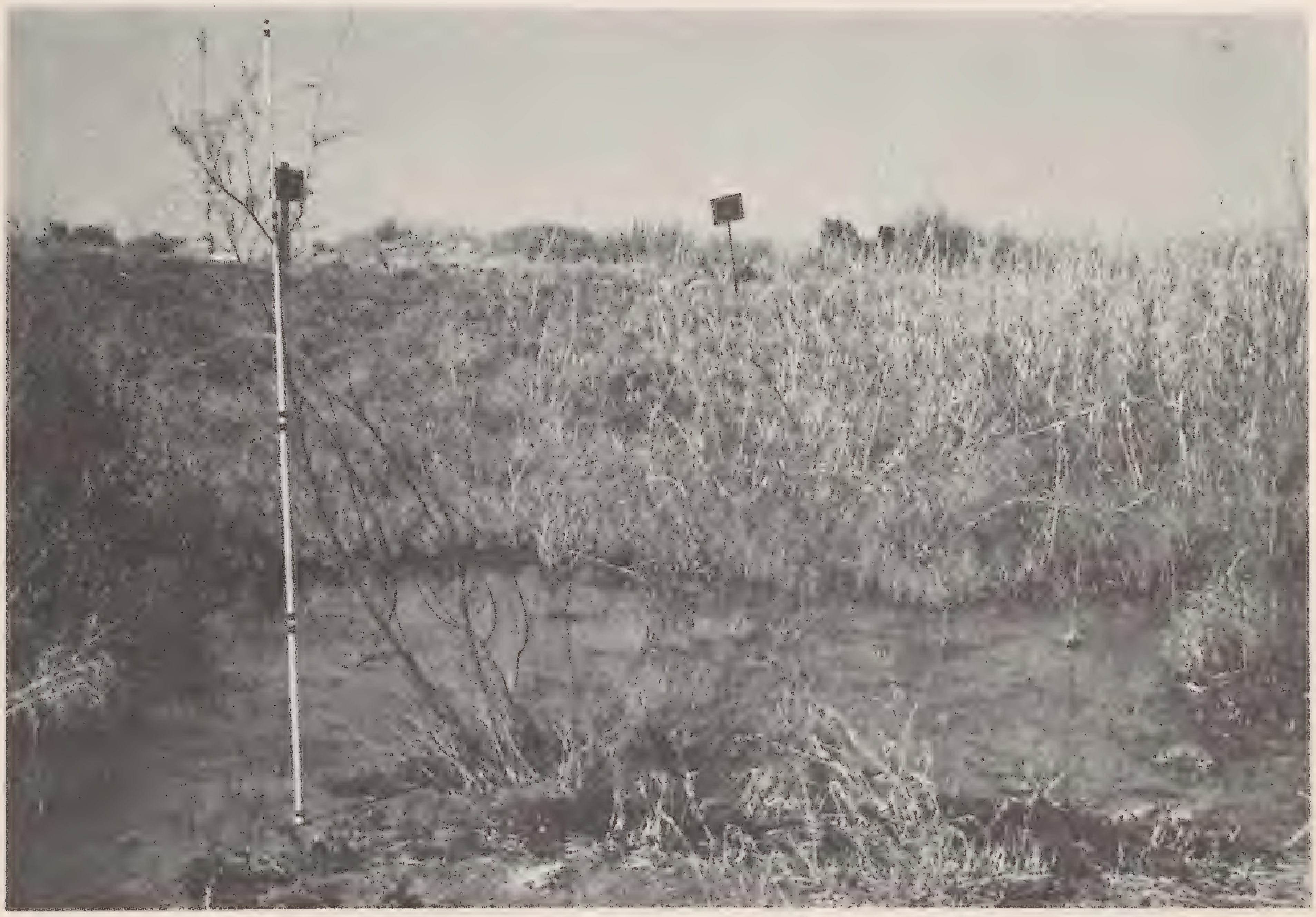

Figure 7.—A typical breeding site of *Culex tarsalis* at Wister: a shallow, grassy depression (site 12) with a recent accumulation of seepage from an irrigation canal.

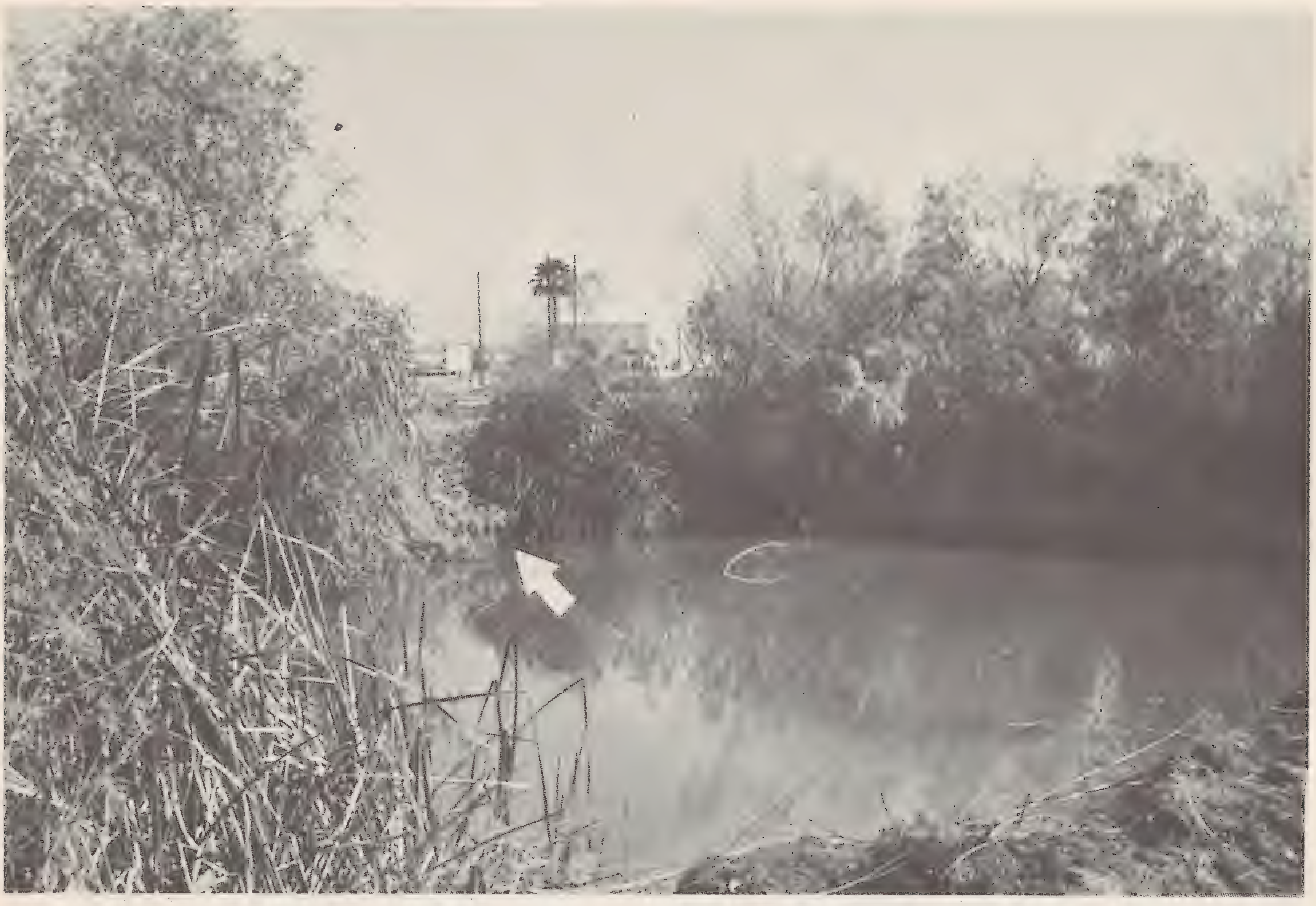

Figure 8.—The sewage oxidation pond at Wister. The arrow indicates the collecting area (site 8) shown in fig. 9.

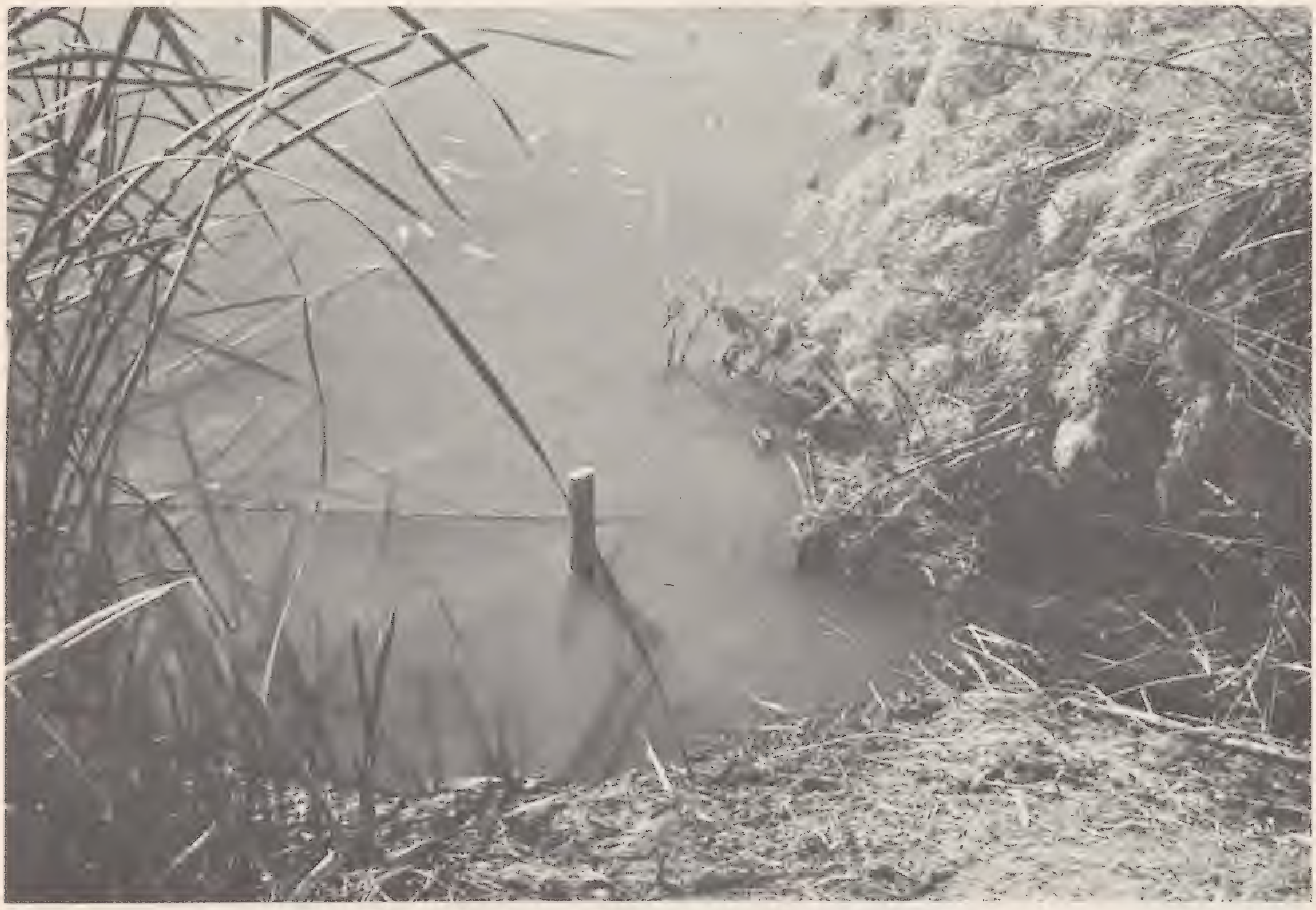

Figure 9.—Site 8 at the edge of the sewage oxidation pond where immatures of *Culex tarsalis* were collected each month. The wooden stake projects approximately 0.25 m above the water surface.

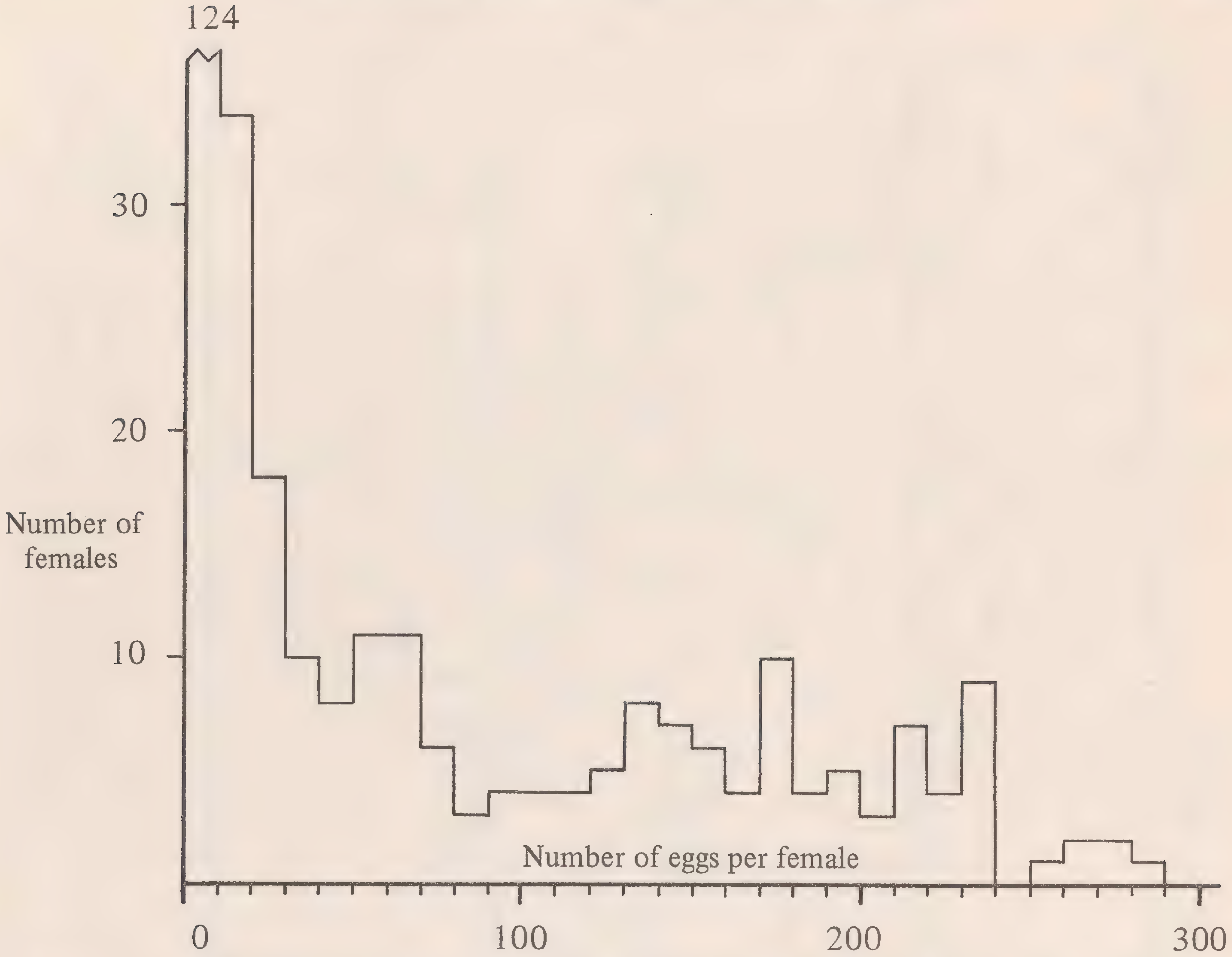

Figure 10.–Frequency distribution of the number of developing eggs (Stages III-V) per female *Culex tarsalis* collected at Wister. September 1967-April 1970.

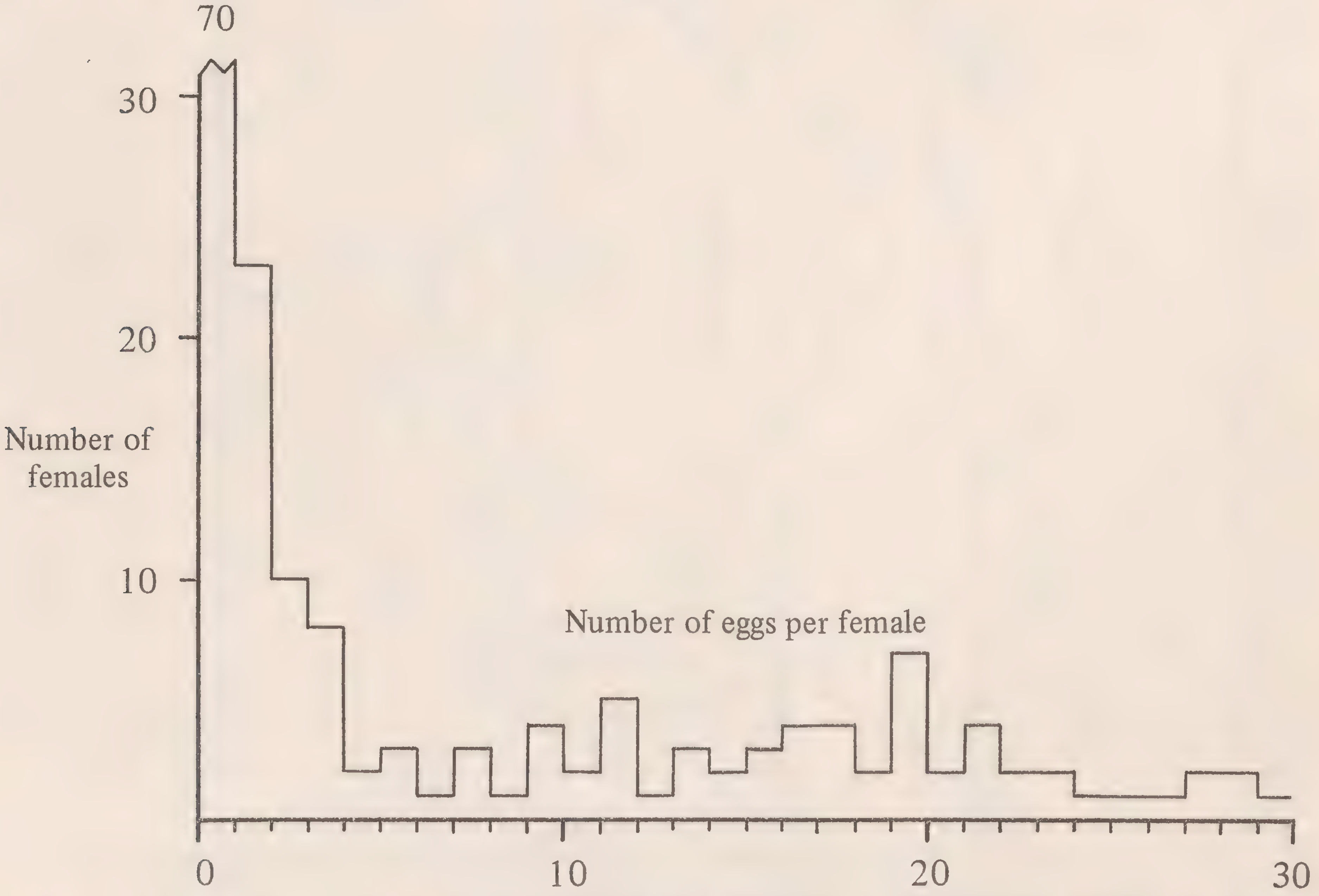

Figure 11.–Frequency distribution of the number of developing eggs per female for those individuals with 30 eggs or less.

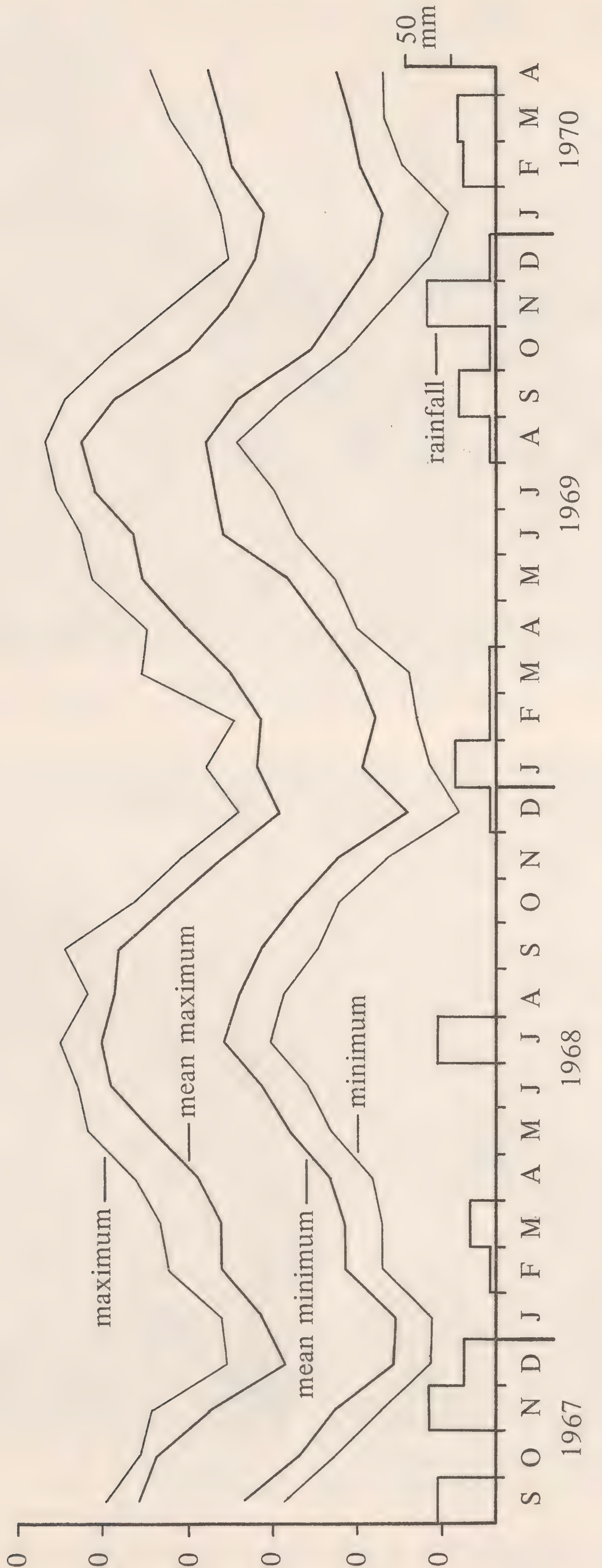

Figure 12.–Monthly mean and extreme temperatures and total monthly precipitation recorded by the Imperial Irrigation District, Imperial, California.

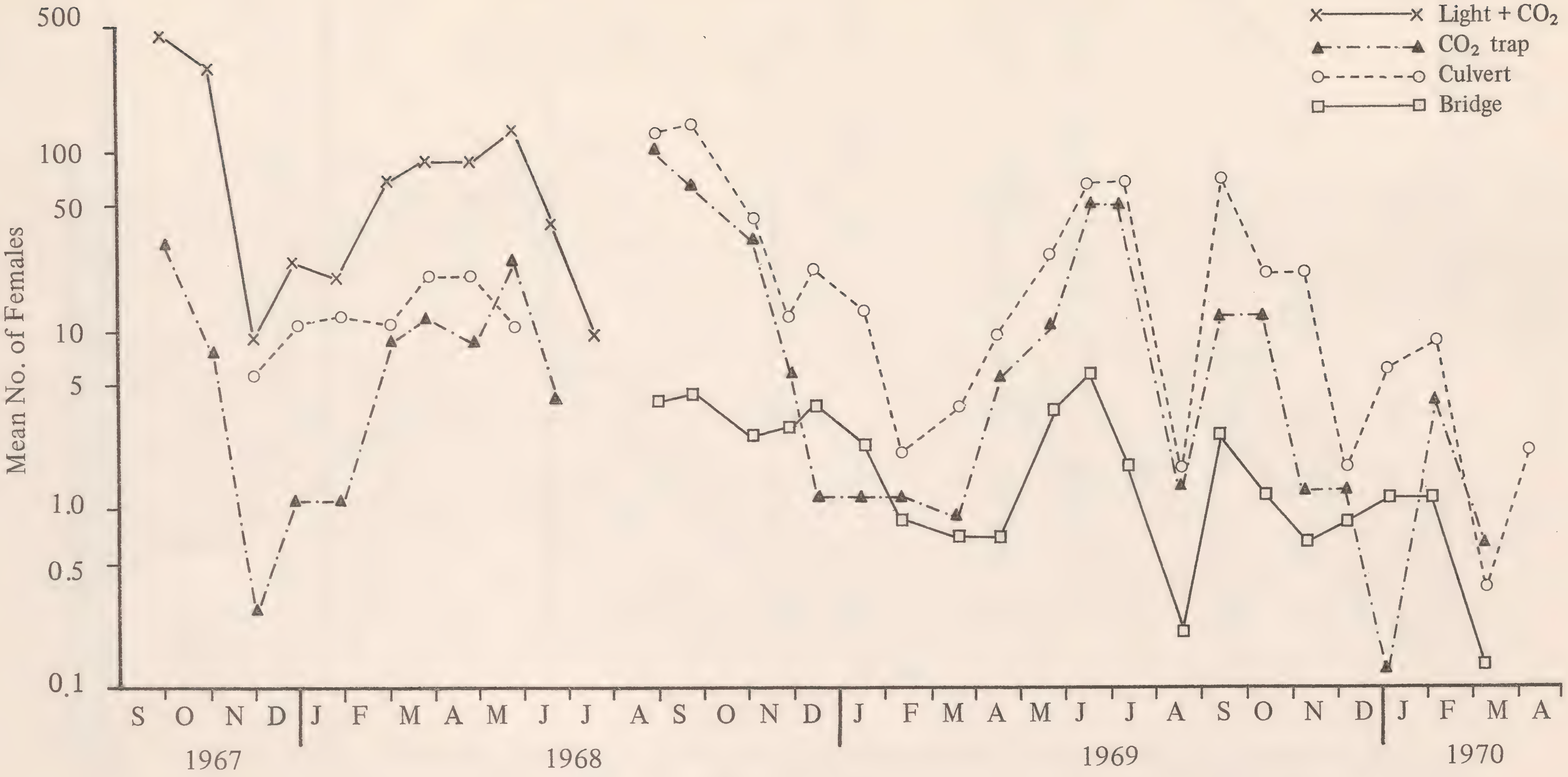

Figure 13.–Numbers of *Culex tarsalis* females collected in light traps with CO_2, CO_2 traps, a culvert shelter (site 6), and 4 bridge shelters (sites 1, 2, 5, 7). Monthly arithmetic means plotted logarithmically.

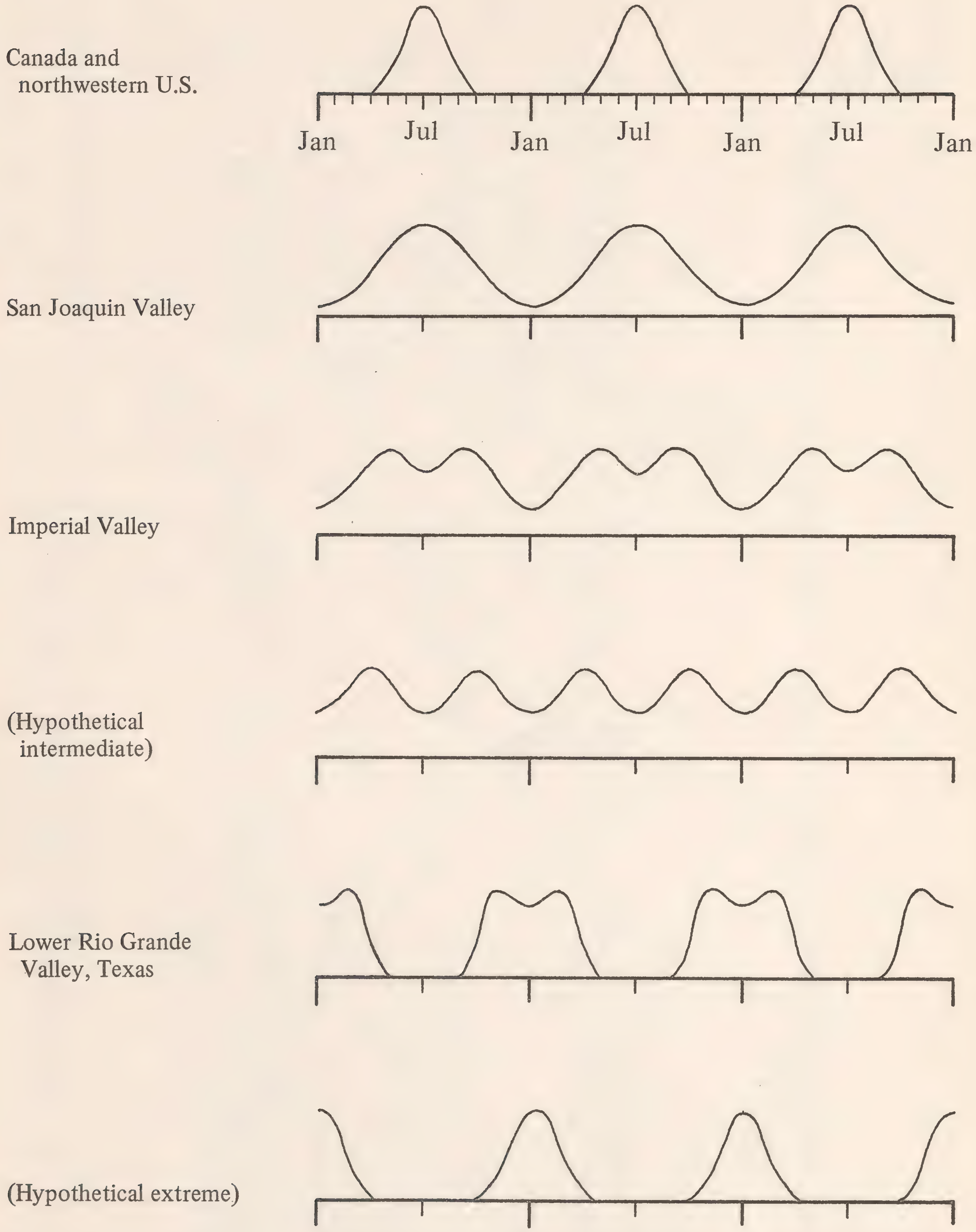

Figure 14.–Diagrammatic representation of the seasonal fluctuations in abundance of *Culex tarsalis* collected in light traps at various latitudes.

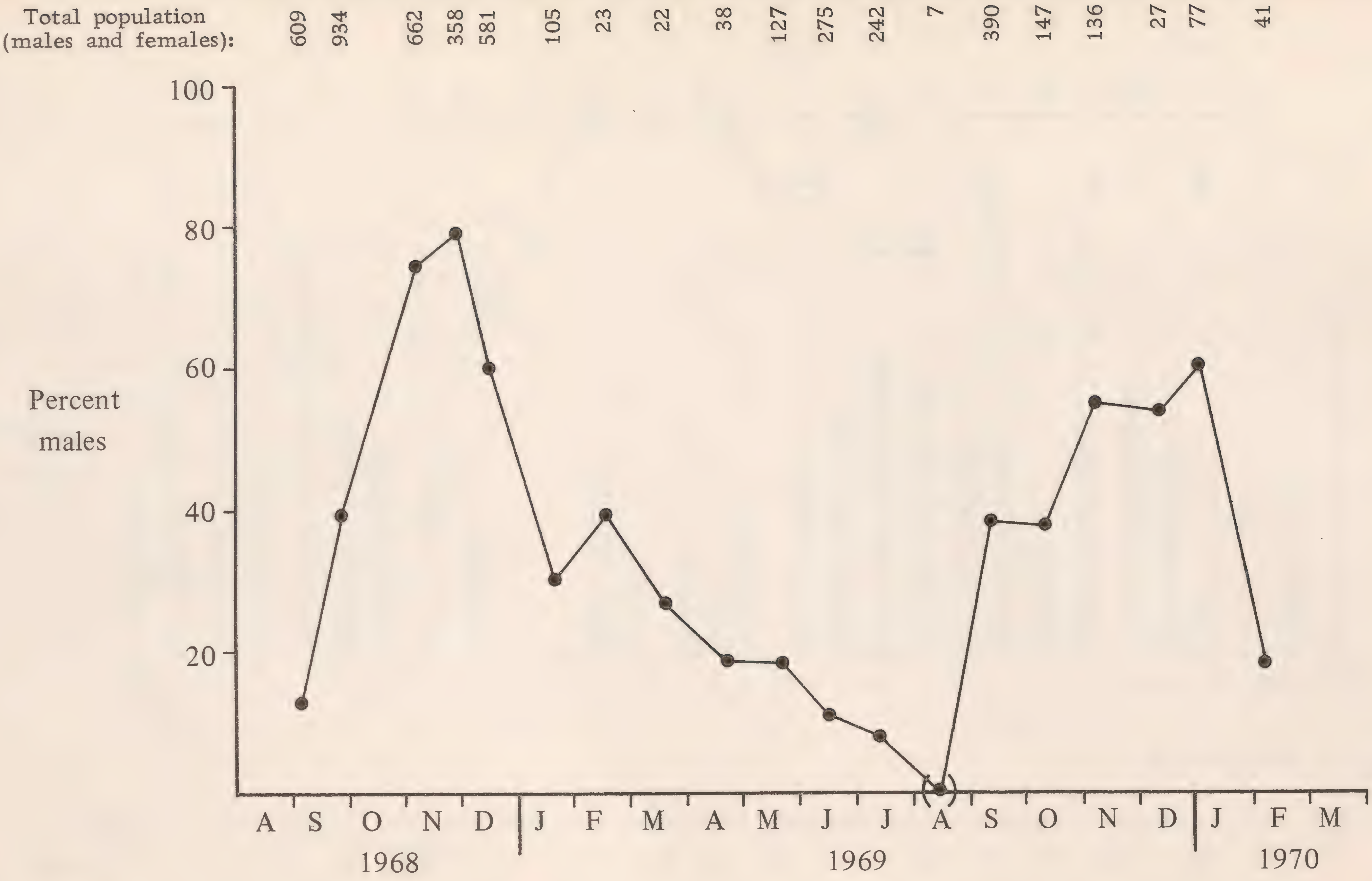

Figure 15.–Monthly percentage of males in the population of *Culex tarsalis* collected in shelters (sites 1, 2, 5-7).

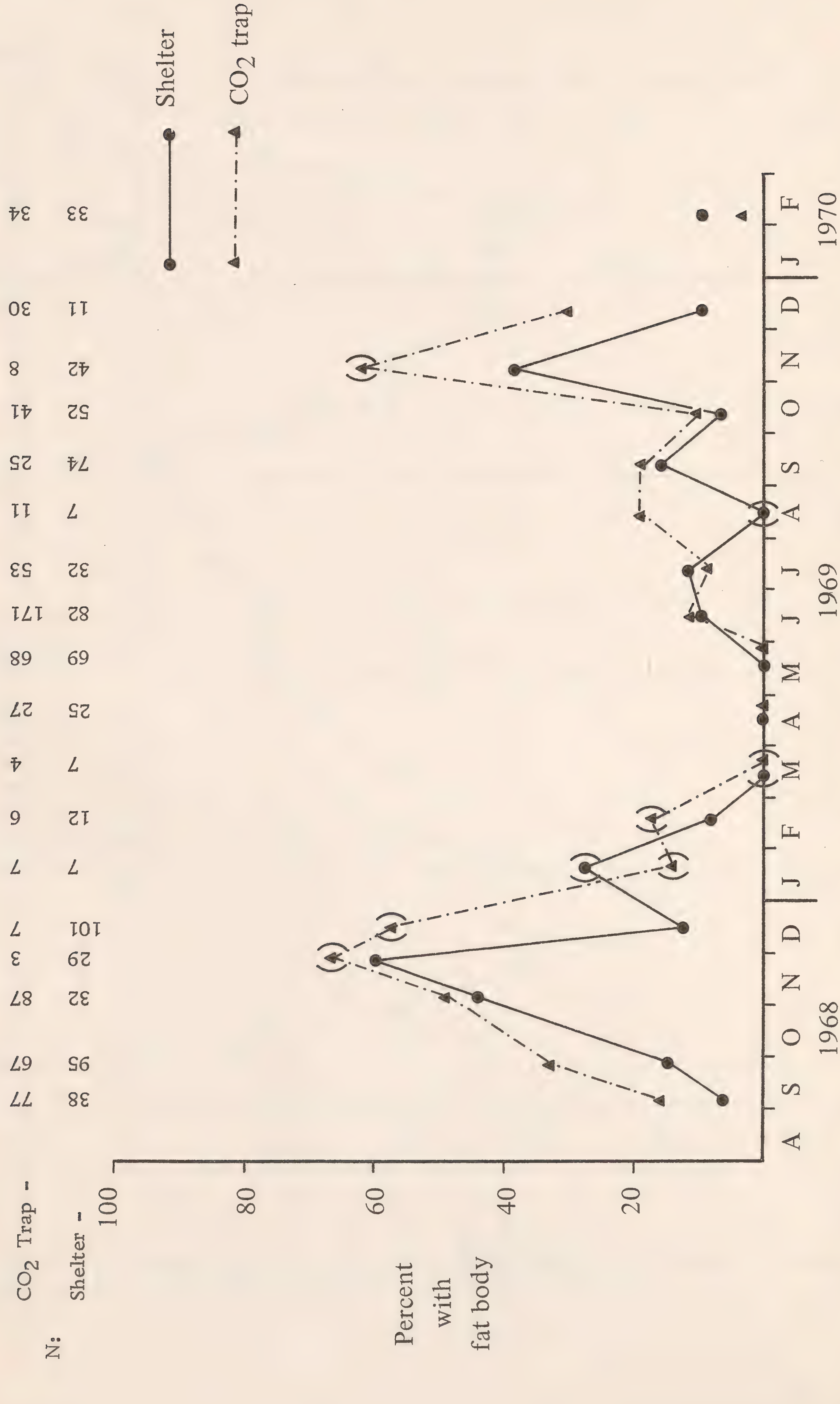

Figure 16.—Fat body. Monthly percentage of female *Culex tarsalis* with Class 3 fat body.

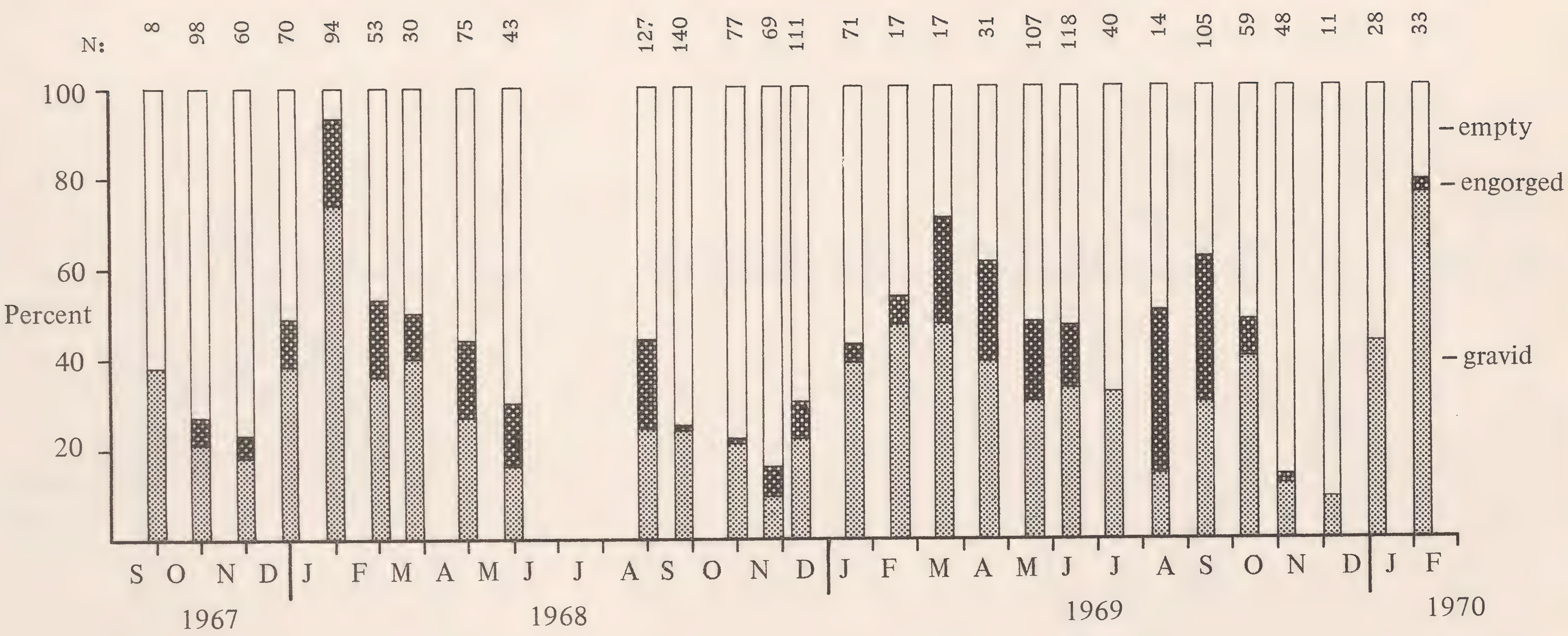

Figure 17.–Monthly percentage of female *Culex tarsalis* empty, engorged or gravid collected in shelters (sites 1, 2, 5-7).

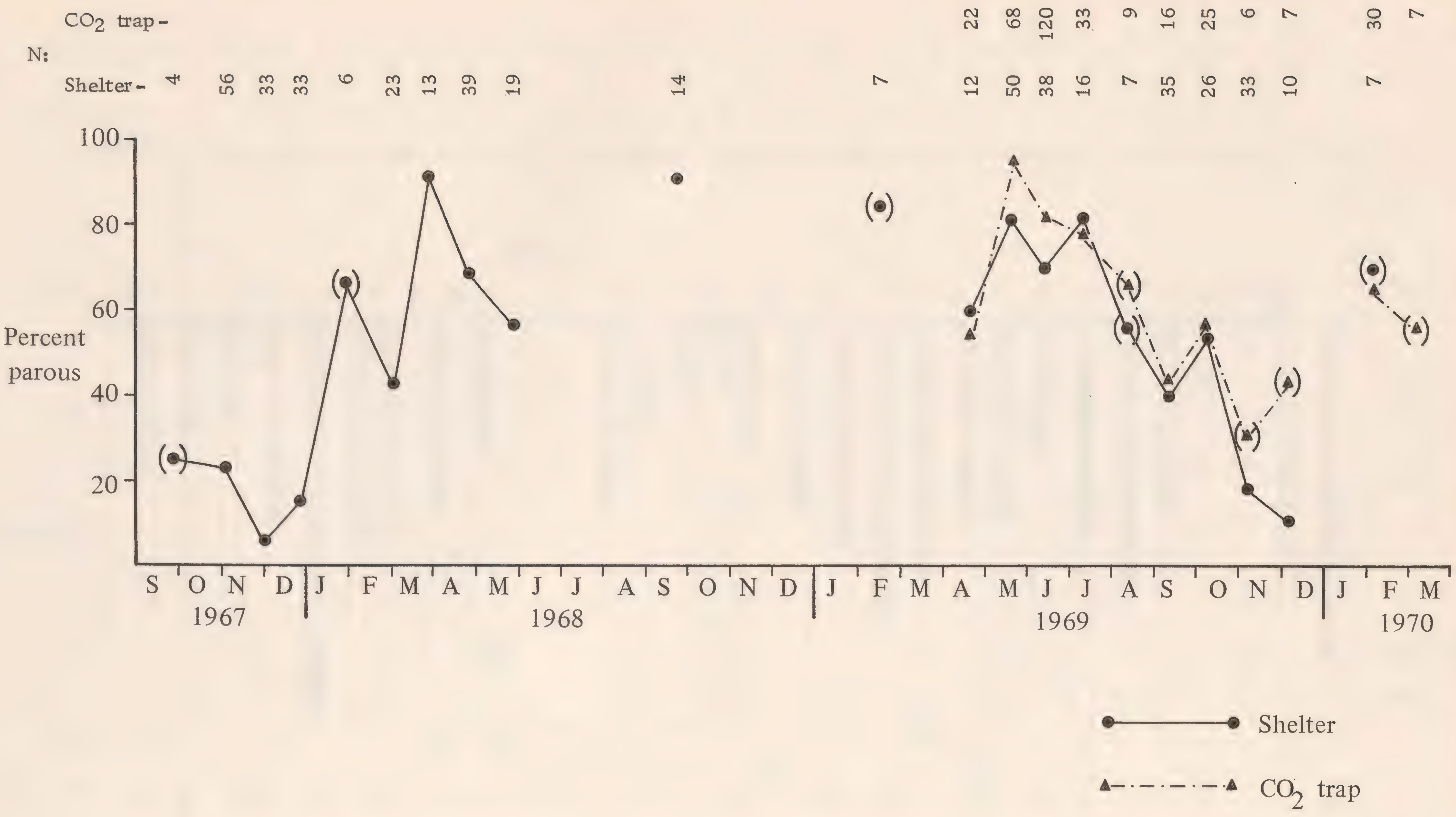

Figure 18.–Parity. Monthly percentage of parous empty *Culex tarsalis* females, collected in shelters and CO_2 traps.